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1939. The comparative toxicity of fluorine in calcium fluoride and in cryolite

J. Nutrition, v. 18, no. 2, Aug. 10

Twelve pairs of growing rats were fed by the paired-feeding technic on a complete diet containing 3.04 p.p.m. of fluorine. The fluorine supplements (calcium fluoride and cryolite) were fed in the drinking water in doses providing equal amounts of fluorine to pair mates and, on an average for the entire experiment, 0.58 mg. of fluorine per kilogram of body weight daily. After 14 weeks of feeding, during which weekly examinations were made of the teeth, the rats were sacrificed, the carcasses dissected into bone, teeth and soft tissue samples and the seventy-two samples thus obtained were analyzed separately for fluorine. No distinction between the fluorine supplements tested was observed with reference to ther effects upon growth, food consumption, the time of appearance of tooth striations, nor the concentration of fluorine in bones, teeth or soft tissues. At the levels fed, the fluorine in calcium fluoride and in cryolite (synthetic) seemed to be equally toxic and equally assimilable. During the experimental feeding period, approximately 59% of the ingested fluorine was retained in the bodies of the growing rats. About 96% of the fluorine retained (at an intake equivalent to 18 p.p.m. of food consumed) was deposited in the skeleton, and the remaining 4% was about equally divided between teeth and soft tissues.

Nutrition — mineral metabolism Rat

Issued by The Wistar Institute, June 12, 1939

UNIVERSITY OF MICHIGAN

RETARDED GROWTH, LIFE SPAN, ULTIMATE BODY SIZE AND AGE CHANGES IN THE ALBINO RAT AFTER FEEDING DIETS RESTRICTED IN CALORIES [1]

C. M. McCAY, L. A. MAYNARD, G. SPERLING AND LeROY L. BARNES
Animal Nutrition Laboratory, Cornell University, Ithaca, New York

FOUR FIGURES

(Received for publication March 7, 1939)

The literature concerning retarded growth and length of life has been reviewed recently (McCay, '39). The present experiments represent an attempt to verify our earlier findings (McCay, Crowell and Maynard, '35) concerning the interrelationships between length of life, the retardation of growth and the effect of long retardation upon the ability to resume growth and attain a normal body size.

Diets used in these studies were made very high in percentage of such constituents as protein, minerals and vitamins in order to insure an adequate ingestion of these essentials. The retardation is effected by the deficiency in the daily allowance of energy in the diet. In the present study every animal was given the same daily allowance of the basal diet. Animals permitted to grow normally were given additional calories.

In none of our studies have we any evidence that the normal animals have their life spans shortened due to the richness of the diet because our values for these fall within the ranges found by Sherman and Campbell ('37), Slonaker ('35) and Drummond et al. ('38).

[1] These studies were started with support from the Snyder Grants made by Mrs. Harry Snyder and were continued under the Rockefeller Grant for Research in Longevity.

The amount of this diet fed to individuals daily was determined by the amount needed to maintain the retarded animals at a stationary body weight. The results of such a regime insure the ingestion of approximately equal amounts of such essentials as protein and minerals, but do not impose any additional burden upon such organs as the kidneys in the case of animals allowed to grow normally. In this case all animals ingested enough protein, vitamins and minerals for growth, but the retarded animals could not grow due to a deficiency of calories.

The composition of the basal diet was the following: cooked starch, 22; cellulose, 2; lard, 10; sucrose, 10; salt mixture (O. and M.), 6; cottonseed oil, 5; yeast, 5; casein, 40.

For vitamin supplements in the present case the control rats that were destined to grow normally were divided into two equal groups. Each member of the first control group was fed a 1% solution of carotene in cottonseed oil in an amount to allow 15 'A' units (A.D.M.A.) per rat per day. In addition, an allowance of $\frac{1}{3}$ gm. of irradiated yeast was given each individual three times weekly. This same allowance of supplements was given each retarded rat. The second control group was fed 3 drops of cod liver oil daily with $\frac{1}{3}$ gm. of dried yeast (not irradiated) fed three times weekly.

The animals allowed to mature at any time were fed additional calories in the form of a mixture of sucrose, cooked starch and lard in the following proportions: 38:57:5.

The plan of the experiment consisted in dividing 106 rats at the time of weaning into two groups, one containing thirty-three members, to serve as a normal control group, and one of seventy-three to be retarded for varying periods. The normal group was then subdivided into two, containing seventeen and sixteen members. The purpose of this subdivision was to have one fed cod liver oil throughout normal life, and the other fed irradiated yeast and carotene for fat-soluble vitamin supplements.

The retarded animals were not subdivided further until the first period of 300 days had passed. At the end of 300

days there were only thirty-eight of the original seventy-three retarded rats alive as well as all of the original thirty-three controls that had been allowed to grow normally.

These losses in the retarded group were due to two failures in the heating system. Under such conditions the normal animals that were well fed could withstand the drastic drop in temperature of the room while the retarded ones that were being kept at maintenance level had little reserve and part of them perished.

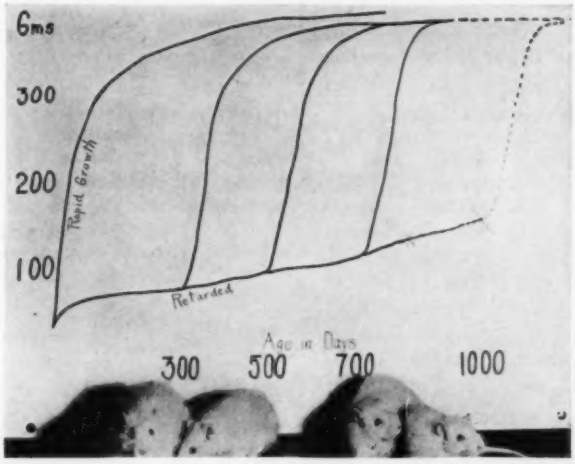


Fig. 1 Projected growth curves showing the plan for the experiment and representatives of the retarded groups at the age of 1000 days.

At the end of 300 days the retarded animals were divided into four groups as nearly equal as possible. One was fed additional calories to permit growth. The other groups were thus assigned at this date to the several periods for resuming growth. These were at the end of 500, 700 and 1000 days. The design of the experiment is shown in figure 1. These curves were drawn in advance and used as a background to photograph typical representatives of each group. In the copy shown in figure 1, the experiment had been in progress

for 1000 days, and all members of the normal group had died while representatives of each of the others were still alive.

The general plan for feeding the retarded animals was to allow them to increase in body weight to the extent of 10 gm. at intervals of about 100 days. The period of maintenance at a fixed body weight is given in table 1. The increase in weight was allowed to take place during a period of a week or 10 days. Fresh liver was fed during this period to allow calories for this growth. Equal amounts of liver were given the controls.

TABLE 1

Period of life of retarded rats during which they were maintained at given levels of body weight

BODY WEIGHT LEVEL *gm.*	AGE OF MAINTENANCE *days*
40	25– 44
60	54– 117
70	130– 228
80	235– 329
90	339– 443
100	450– 548
110	555– 688
120	696– 716
130	731– 822
140	836– 892
150	904– 955
160	963–1004

In assembling data upon such values as ultimate body size for these groups certain individuals had to be excluded, such as members of the group retarded for 1000 days that died before attaining this age. These individuals can be included, however, in considering such factors as disease that caused death or the degree of calcification of the arteries and organs.

The data concerning body weight are in table 2. In general retarded animals were never able to attain the body weights of those that grew normally. This indicates that even in retardation for a period of 300 days there is a permanent stunting effect upon the rat body. In general, the females tend to be lighter in weight than the males even after they

have been kept at the same level during long periods of retardation.

The length of life for each individual is given in table 3. In the groups that grew to maturity normally the death rate of the males is about that anticipated from such a limited number. The females in these groups died prematurely, however. From earlier experience about 20% of them should have exceeded 1000 days, but all of them were dead before this time.

TABLE 2

Maximum weights in grams by groups and subgroups

GROUP	SEX	MEAN BODY WEIGHT AT TIME OF REALIMINATION	MEAN MAXIMUM WEIGHT ATTAINED	MEAN AGE OF ATTAINING MAXIMUM WEIGHT	MAXIMUM WEIGHT ATTAINED BY ANY INDIVIDUAL	NUMBER OF ANIMALS CONSIDERED
				days		
Normal growth	♂		450±15	586±24	555	9
Fed carotene	♀		321±16	446±32	416	8
Normal growth	♂		445±17	543±47	567	8
Fed cod liver oil	♀		372±24	572±45	536	8
All retarded	♂		327±18	832±25	485	15
	♀		260±10	846±42	365	16
Retarded 300 days	♂	79	415±15	709±38	443	4
	♀	79	244±11	603±70	300	5
Retarded 500 days	♂	105	371±36	801±32	485	4
	♀	104	298±16	849±20	350	5
Retarded 700 days	♂	148	287±27	844±36	390	4
	♀	138	247±30	985±64	365	4
Retarded 1000 days	♂	179	209±22	1023±20	270	3
	♀	183	232±21	1164±37	263	2

Formula used: $PE_m = 0.6745 \sqrt{\frac{\Sigma fd^2}{n(n-1)}}$ $(f = 1)$.

The mortality curve for those fed cod liver oil is more nearly normal than for those fed carotene and irradiated yeast as sources of vitamins A and D. None of these data indicate any unfavorable effect of cod liver oil when fed during the entire life span.

The retarded groups all had members alive when the last of the normal controls had perished. These retarded groups

also contained members that far exceeded the normal life expectancy.

The period of adult life tends to be much shorter after long retardation. A brief table (4) will show this. The period of life remaining after maturing becomes progressively less as the length of the retarded period increases.

TABLE 3

Life span of individuals in days (those living over 300 days). The figures below the line represent those rats still alive when the last control died

NORMAL GROWTH FED CAROTENE		NORMAL GROWTH FED C.L.O.		RETARDED 300 DAYS		RETARDED 500 DAYS		RETARDED 700 DAYS		RETARDED 1000 DAYS	
♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
377	475	308	404	805	535	366	793	772	406	336	815
643	527	439	624	815	598	721	1008	782	697	859	846
673	536	506	636	822	711	856	1028	915	867	1004	1152
703	555	627	646	1018	1007	986	1043	1025	1045	1086	1320
713	570	668	722		1183	1103	1078		1134	1127	
720	635	809	808						1320		
767	650	869	844								
784	682	886	965								
896											

TABLE 4

Length of life after attaining maturity

RETARDED IN DAYS	MEAN AGE ATTAINED BY THOSE ALIVE AT 300 DAYS	TIME LIVED AFTER COMPLETING GROWTH *days*
300	835	535
500	895	457
700	896	253
1000	949	138

As a whole the effect of retardation of growth is similar to that obtained previously. A much higher per cent of the rats can attain ages that exceed 1000 days if they have been subjected to retardation. Thus from the retarded groups six males exceeded an age of 1000 days. These came from an original group of only thirty-eight males. No data are available to permit an estimate of the number of males needed in a normal population to secure this number at this age.

The line was drawn in table 3 to indicate the number alive in the retarded groups at the time of death of the last of the normal-growth animals. Retardation permits a higher per cent of animals to attain old age if the diet is qualitatively complete in other respects. This does not conflict with the data of Sherman and Campbell ('37).

These results in combination with those of the earlier report (McCay, Crowell and Maynard, '35) indicate that the method of retardation affords a useful technic for studying aging. Animals that are relatively very old become available for study. Furthermore, the parts of the body that age in a

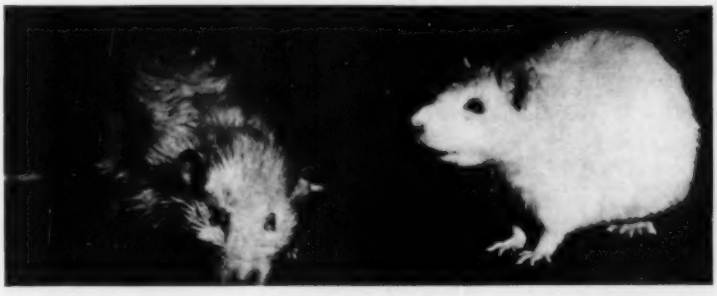


Fig. 2 Age 964 days. This photograph was taken the last day of life for the last of the controls on the left and 36 days before the retarded rat on the right was allowed to mature. Note the contrast in degree of senescence.

normal animal can be compared with those of retarded animals that are destined for a much longer span. This affords a tool for investigating the systems that break down in the normal process of aging.

The condition of the hair of the retarded animals tended to remain fine in contrast to the coarseness in the case of those that grew normally. This is evident in the photograph in figure 2. The retarded animal on the right in the picture was allowed to mature at 1000 days of age and lived nearly a year after this photograph was made.

The weights of the organs in table 5 indicate that the ultimate body size attainable decreases in proportion to the

TABLE 5

Effect of long retardation followed by realimentation upon the weight of organs

	NORMAL GROWTH FED CAROTENE		NORMAL GROWTH FED C.L.O.		ALL RETARDED		RETARDED 300 DAYS		RETARDED 500 DAYS		RETARDED 700 DAYS		RETARDED 1000 DAYS	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Mean body weight in grams less organs and gastro-int. tract	235	184	248	173	196	128	220	110	214	139	177	133	123	140
Mean weight of liver in grams	10.6	9.2	11.4	11.6	10.7	8.2	10.4	8.5	12.6	8.8	10.9	7.2	8.1	8.2
Mean weight of kidneys in grams	2.6	2.4	3.0	2.5	2.2	1.8	2.4	1.8	2.5	2.0	2.3	1.8	1.6	1.5
Mean weight of spleen in grams	1.4	1.3	1.0	1.2	0.9	1.0	1.2	1.0	0.8	1.0	0.9	0.9	0.6	0.7
Mean weight of heart in grams	1.6	1.4	1.3	1.4	1.3	1.2	1.4	1.4	1.4	1.1	1.2	1.0	1.1	1.6
Mean body length nose to anus (cm.)	21.4	20.1	22.3	18.5	20.2	18.6	21.2	18.6	20.6	19.5	20.0	17.7	18.3	18.0
Number of animals	7	9	8	8	15	16	4	5	4	5	4	4	3	2

period of retardation. There is probably no significant difference between the two normal groups. In general our findings accord with those of Jackson ('37) and co-workers in showing that retardation leads to a permanent decrease in the size of the body and its parts.

Bone growth

After careful dissection bones were allowed to air dry and used for determining the size and density. In the normal groups the ranges in density were the following: male humerus, 1.108 to 1.362; femur, 0.937 to 1.286; female humerus, 1.200 to 1.407; femur, 1.062 to 1.275. The bones of the males tended to become less dense with age while there was no change in those of the females up to 965 days.

A condensed summary of bone data for all groups is presented in table 6. As far as bones can serve as criteria these data indicate that the loss of the ability to attain a maximum body size is progressive with the period of retardation.

The permanent decrease in the body size after such a long period of retardation as 1000 days, even among the animals that could resume growth, was between 10 and 20% below the normal as far as the bone data indicate. This is illustrated in figure 3. Body length data also indicate that the better of the group retarded for this long period attained a length of about 85%. From the organ data, such as the weight of the organs or of the 'empty' body, the better animals seem to have attained a size of about two-thirds the normal. Such data are subject to considerable variation, however, because the animals were both old and diseased at the time of death.

The density of the bones from retarded animals is low. This is partly due to the great age at which these animals die. The mean density for both sexes of the retarded animals was 1.21 for the humeri. In the case of retarded animals there is considerable variability and in animals over 1000 days of age the density of the humerus varied from less than 1 to more than 1.3. No explanation can be offered for the absence of sex differences in the retarded groups except that

TABLE 6

Changes in the size and weight of bones after retardation followed by growth

DIET	SEX	BONES	LENGTH	WEIGHT (AIR DRY)	WEIGHT (OVEN DRY)	DENSITY (AIR DRIED)
			cm.	mg.	mg.	
Normal	♂	Humerus	3.0	341	321	1.26
growth	♀	Humerus	2.7	235	268	1.35
'carotene'	♂	Femur	3.9	702	666	1.10
	♀	Femur	3.5	586	552	1.22
Normal	♂	Humerus	2.9	335	316	1.26
growth	♀	Humerus	2.7	263	250	1.33
'cod liver oil'	♂	Femur	3.8	691	644	1.10
	♀	Femur	3.5	540	509	1.20
Retarded	♂	Humerus	2.8	309	289	1.26
300 days	♀	Humerus	2.5	238	222	1.21
	♂	Femur	3.7	633	597	1.18
	♀	Femur	3.3	453	428	1.13
Retarded	♂	Humerus	2.6	264	248	1.26
500 days	♀	Humerus	2.6	237	222	1.20
	♂	Femur	3.5	535	502	1.18
	♀	Femur	3.4	471	442	1.13
Retarded	♂	Humerus	2.6	257	238	1.18
700 days	♀	Humerus	2.5	202	189	1.24
	♂	Femur	3.6	553	522	1.15
	♀	Femur	3.1	391	368	1.11
Retarded	♂	Humerus	2.5	219	212	1.15
1000 days	♀	Humerus	2.4	201	188	1.18
	♂	Femur	3.2	384	406	1.06
	♀	Femur	3.1	373	348	1.03

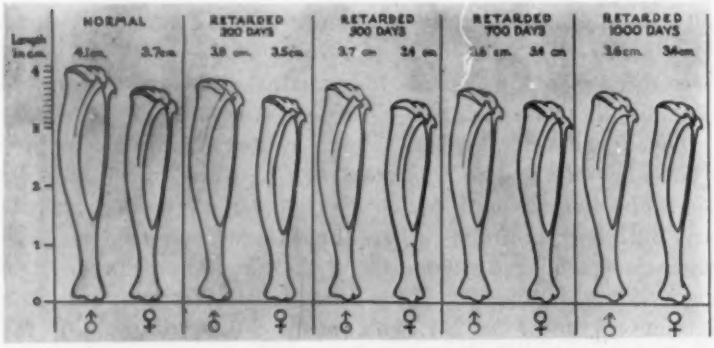


Fig. 3 The effect of retardation and realimentation upon the ultimate size of bones in rats of opposite sex.

they were kept at the same body weights. In both the previous report and this one the life span of the opposite sexes seemed to be the same in the case of retarded animals.

Bone growth after long retardation

The growth of the bones in rats that had been retarded for 700 days and more was followed by making x-ray photographs at frequent intervals. From these the length of the

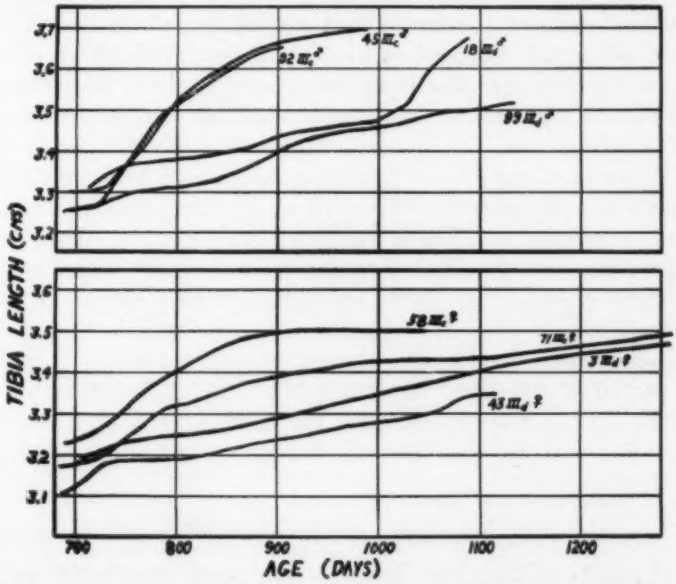


Fig. 4 Growth of the tibia in rats retarded for 700 and 1000 days from measurements of x-ray photographs. Curves III c are for animals retarded 700 days. Those marked III d were allowed to resume growth after 1000 days.

bones such as the tibia could be measured. Typical data from these measurements are plotted in figure 4. The upper curves for each sex show the rates at which the bones responded when the rats were allowed to grow after 700 days of age. After 700 days all retarded animals responded promptly when allowed enough calories for growth. Although the opposite

sexes were approximately the same in body weight at the beginning of this period the bones of the females were smaller. The bones of the males respond to the realimentation more promptly than those of the female. The bones of the male retain the power to grow larger than those of the female.

Of the rats retarded for 1000 days only three males and two females were alive at the date set for realimentation. Curves for four of these animals are marked III-d in figure 4. The first characteristic of these is the slow, regular increase in the length of the bones that was taking place even when the individual was held at a constant body weight. Even at these extreme ages the bone retains its power to forge ahead in growth while the other parts of the body remain stationary or shrink.

After these rats were fed adequately, part seemed able to accelerate the growth of the bone while others seemed to have lost this power. This is the first evidence that there is a time limit for retardation beyond which the body can no longer resume growth. At 1000 days some animals seem to retain this power of growth and others seem to have lost it. This is not the case in the earlier study when retardation exceeded a period of 900 days. Even after this extreme retardation of 1000 days in the present case the male is still able to grow to a larger body size than the female, as far as the skeleton indicates.

MAINTENANCE REQUIREMENTS FOR RETARDED GROWTH RATS

Jackson ('37 a) has discussed the requirements for maintenance of rats held at a constant body weight, recently. The results of the present study confirmed those of the earlier one inasmuch as the female at most levels needed more calories for maintenance than the male.

SUMMARY

Rats were retarded in growth for periods of 300, 500, 700 and 1000 days before being allowed to grow to maturity. Members of each of these groups were alive when the last of

the control groups had died at an age of 965 days. Retardation of growth by diets, complete except for calories, affords a means of producing very old animals for studying aging. Animals that are retarded for even 300 days can never become as large as those that mature normally. After 1000 days of retardation only part of the rats were able to resume growth when adequate energy was allowed in the diet. Even at this extreme period the male tended to grow to a larger size than the female. The growth of the bones in rats retarded for 700 and 1000 days was followed by means of x-ray photographs. The maintenance of a constant body weight in this period of old age does not check the growth of the bones. These increase slowly and respond to realimentation in all cases after 700 days of retardation but in only part of the cases after 1000 days.

LITERATURE CITED

DRUMMOND, J. C., A. Z. BAKER, M. D. WRIGHT, P. M. MARRIAM AND E. M. SINGER 1938 The effects of life long subsistence on diets providing suboptimal amounts of the vitamin B complex. J. Hygiene, vol. 38, p. 356.

JACKSON, C. M. 1937 Recovery of rats upon refeeding after prolonged suppression of growth by underfeeding. Anat. Rec., vol. 68, p. 371.

———— 1937 a The food intake of young rats held at nearly constant body weight by restriction of the dietary protein. J. Nutrition, vol. 13, p. 669.

MCCAY, C. M., M. F. CROWELL AND L. A. MAYNARD 1935 The effect of retarded growth upon the length of the life span and upon the ultimate body size. J. Nutrition, vol. 10, p. 63.

MCCAY, C. M. 1939 Problems of ageing (edited by E. V. Cowdry). Williams and Wilkins Co., Baltimore. Chap. 21.

SHERMAN, H. C., AND H. L. CAMPBELL 1937 Nutritional well-being and length of life as influenced by different enrichments of an already adequate diet. J. Nutrition, vol. 14, p. 609.

SLONAKER, J. R. 1935 The effect of different percentages of protein in the diet on bachelor and virgin rats. Am. J. Physiol., vol. 113, p. 159.

CHEMICAL AND PATHOLOGICAL CHANGES IN AGING AND AFTER RETARDED GROWTH [1]

C. M. McCAY, G. H. ELLIS, LEROY L. BARNES, C. A. H. SMITH AND GLADYS SPERLING

Animal Nutrition Laboratory, Cornell University, Ithaca, New York

FOUR FIGURES

(Received for publication March 7, 1939)

In the preceding paper (McCay, Maynard et al., '39) a summary was given of the results of retarding rats for periods varying from 300 to 1000 days before permitting them to grow to maturity. In this report are incorporated the data concerning the changes in the chemical composition of some of the organs and a brief consideration of some of the pathology involved in these same animals.

CHANGES IN THE CALCIUM CONTENT OF THE EYES

The method used for calcium was that described by Ellis ('39). The tissues to be analyzed, chiefly eyes, were first dried to a constant weight at 105°C. All values were expressed in terms of per cent of the dried weight. Up to about an age of 1000 days the calcium of the eye varies between 0.019 and 0.092%. After 1000 days of age these values range between 0.079 and 1.67%.

In table 1 these data are assembled according to the age of the rat at death without regard to its previous treatment. The great increase in the calcium of the eyes in old age is more marked than that found in any other species. This literature has been summarized previously (McCay, '39).

[1] These studies were started with support from the Snyder Grants made by Mrs. Harry Snyder and were continued under the Rockefeller Grant for Research in Longevity.

The data for the calcium of the eye were studied from many angles. Retarded rats tended to live longer and the level of calcium was higher at the time of death. The eye calcium seems to increase in proportion to the time a rat has lived without regard to the retardation of growth. No correlation could be found between the level of calcium in the eye and the degree of calcification of such organs as the kidneys. There was no relation to the density of the bones.

In two cases where it was possible to dissect the lens, the calcium was lower than in the rest of the eye. Calcium was determined in the aortas from a few very old animals. On

TABLE 1

Age and calcium content of the entire eye

AGE GROUP	NUMBER OF RATS	MEAN PER CENT Ca
days		
506– 555	5	0.034
627– 697	8	0.034
703– 720	3	0.058
805– 886	9	0.043
965–1078	10	0.238
1103–1183	5	0.499
1320	2	1.078

a dry basis these varied from 0.27 to 6.0%. No relation was found to the eye calcium.

THE TEETH OF RATS IN THE COURSE OF AGING AND AFTER RETARDATION

The condition of the teeth in rats is important in life span studies because they may serve as foci of infection or they may modify the consumption of food due to pain.

The jaws and teeth were examined under low magnification by a practising dentist. X-ray photographs of the jaws and teeth were also made.

The females seemed to suffer more consistently from the breakdown of teeth than the males. This was especially true in the normal animals. In both of these groups the mean age of the males exceeded that of the females. As a whole the

retarded rats showed increased destruction of teeth, but considering their advanced age they were remarkably free.

In the first control group fed carotene and irradiated yeast as sources of fat soluble vitamins, one male animal at the age of 896 days had lost two lower molars. Another female had a left molar that was carious to the gum line with the roots retained. In another female, dead at an age of 527 days, the the upper molars were normal while all but one of the lower molars had been completely destroyed. Similar conditions were noted in the second control group. One of the males in

TABLE 2

The effect of aging and retardation upon the condition of the teeth at death

GROUP	NUMBER AND SEX	MEAN AGE	NUMBER OF RATS WITH INTACT TEETH	PER CENT OF TEETH MISSING OR CARIOUS IN THE GROUP
Control	6 females	597	2	26
'carotene'	8 males	679	2	13
Control	8 females	600	0	33
'C. L. O.'	8 males	639	2	25
Retarded	4 females	717	1	23
300 days	4 males	865	1	23
Retarded	4 females	985	0	35
500 days	4 males	915	0	17
Retarded	4 females	935	1	23
700 days	4 males	873	0	25
Retarded 1000 days	3 females	937	0	36
or less	4 males	1019	1 (almost)	29

this group which had died at an age of 886 days had all teeth badly abraded. In one member of the group retarded for 300 days and dead at an age of 1007 days, all of the lower molars were gone, while the upper molars were normal except for loss of the alveolar process. However, in one case of those retarded for 1000 days, a male had nearly an intact set of teeth.

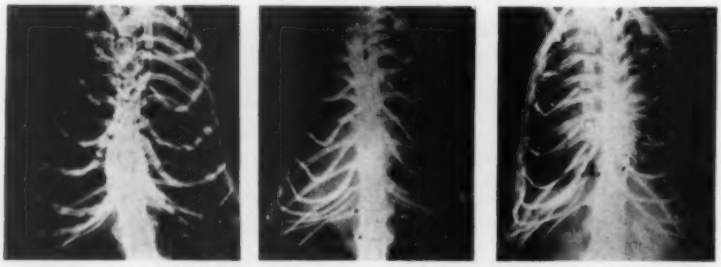
These data are summarized in table 2.

The variability among rats seems fully as great as that found in human beings.

THE CALCIFICATION OF COSTAL CARTILAGES

X-ray pictures were used to determine the degree of calcification of the costal cartilages. In animals dying at similar ages, calcification was much more marked in the control than in the retarded animals. Retardation of growth delays this calcification.

In figure 1 a is shown the oldest control, age 965 days, in figure 1 c a rat retarded for 1000 days, age 1086 days, and in figure 1 b another that was retarded for 1000 days but lived to an age of 1320 days. The greatest difference here is



a	b		c	
Control, Age 965 days	Retarded Age	1000 days 1320 days	Retarded Age	1000 days 1086 days

Fig. 1 Heavily calcified costal cartilages of 'normal growth' rat on the left (a) contrasted with 'retarded' ones on the right (b and c).

seen between the control and the 1086-day-old rat. The 1320-day-old rat shows more calcification of the costal cartilages than the 1086-day-old rat, but not as much as the control.

If calcification is an index of physiological aging of cartilage, it then appears that the retardation affects this process.

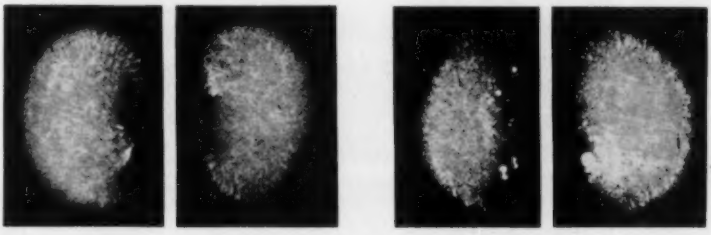
THE DEGREE OF CALCIFICATION OF THE HEART, AORTA AND KIDNEYS OF NORMAL AND RETARDED RATS

Observations were only made on those rats that lived more than 700 days. X-ray pictures were made, at the time of autopsy, of ten control and thirty-two retarded rats. The pictures were made with a tungsten target tube at a peak

voltage of 35 to 40 K.V. No filter other than the glass wall of the tube was used. The film was covered with two thicknesses of black paper. The calcification of the tissue of the kidneys, hearts and aortas will be discussed.

Kidneys

The x-ray stereograms of the kidneys make possible an estimation of the relative extents of calcification in the different parts of the kidney. The calcification, as illustrated in figures 2 a and 2 b, frequently extends through the pelvis, pyramid and medulla, but its appearance in the cortex is infrequent and never extensive.



a		b	
Retarded	500 days	Retarded	700 days
Age	1008 days	Age	1025 days

Fig 2 Extensive calcification in the kidneys of two retarded rats.

Table 3 was prepared from a study of the stereograms of the kidneys. It is not possible in most cases to distinguish calcium deposits in the pyramid from those in the pelvis so these regions of the kidney are considered as one. The extent of the calcification in each of the three regions was estimated on a basis of 0 to 3, 0 indicating no calcification and 3 indicating the most extensive case.

All of the control rats on which these observations were made died in the age range from 713 to 965 days. Twelve of the retarded rats died in the age range, 711 to 915 days. An examination of table 3 will show that both the incidence and

extent of calcification of the kidneys of the retarded rats are much higher than that of the controls of approximately the same age.

Eighteen of the retardeds died in the age range of 986 to 1320 days. Their kidneys were also extensively calcified but no more extensively than those of the younger group of retardeds.

TABLE 3

Incidence and extent of calcification in kidneys

NUMBER OF RATS IN GROUP	AGE AT DEATH	CALCIFICATION					
		Pelvis and pyramid		Medulla		Cortex	
		Incidence	Extent	Incidence	Extent	Incidence	Extent
	days	%		%		%	
8 control	713 to 965	0	0	31	0.3	0	0
12 retarded	711 to 915	96	1.6	87	1.6	21	0.2
18 retarded	986 to 1320	95	1.7	86	1.7	17	0.2

Aortas

For making the x-ray pictures of the aortas, they were split longitudinally and laid flat on a piece of paper. Many of the aortas of the retarded rats showed extensive calcification. Examples of some of the more extensively calcified aortas are shown in figure 3.

Table 4 shows the incidence and extent of the calcification of the aortas of thirty-two retarded rats and ten controls. The incidence and averge extent of calcification are both markedly higher in the case of the retardeds, and as in the case of the kidneys the last eighteen retardeds that died are not appreciably different than the fourteen retardeds preceeding them. However, it should not be concluded from this that the calcification of the aortas parallels the calcification of the kidneys. Table 5 shows the incidence and extent of calcification of the aortas in relation to the time of retardation. The lowest incidence and the least extensive calcification of aortas is in the case of the controls, which were not

retarded at all. The highest incidence and the most extensive calcification is in the case of those rats retarded 1000 days. It should also be noted that there is a progressive increase in the incidence and extent of the calcification with increasing retardation. A similar comparison of calcificaton of kidneys

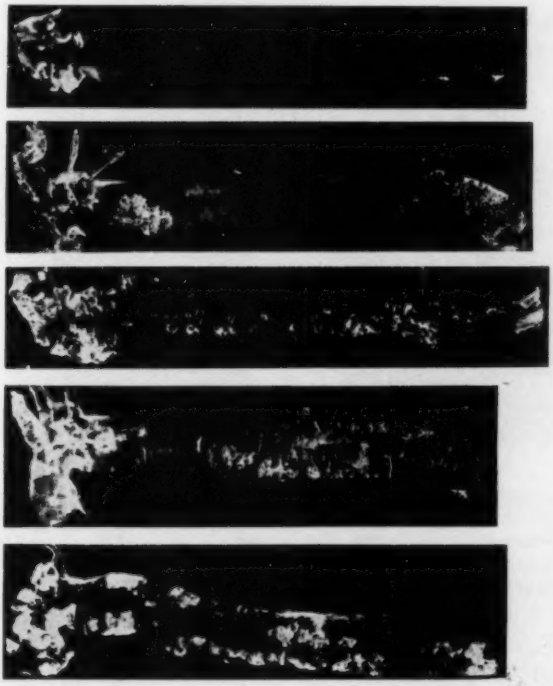


Fig. 3 : Aortas of retarded rats showing calcification in the region of the arch. The lower pictures show calcification over the entire length.

shows no such effect within the retarded groups. The kidneys of the rats retarded for 300 days shows just as extensive calcification as those retarded for 1000 days. A preliminary report of the calcification of the aortas of rats was made by Hummel and Barnes ('38).

Hearts

X-rayed stereograms of the hearts of all of these rats were also made. Unfortunately, the auricles were removed from some of the hearts before the stereograms were made. Since some calcification seems to occur near the base of the heart, we cannot make as reliable comparisons as were made for kidneys and aortas. It may be said however, that the

TABLE 4

Calcification in aortas

NUMBER OF RATS IN GROUP	INCIDENCE OF CALCIFICATION OF AORTA	AVERAGE EXTENT OF CALCIFICATION OF AORTAS	RANGE OF AGES AT DEATH
	%		*days*
10 controls	20	0.2	713 to 965
14 retardeds	57	1.1	711 to 915
18 retardeds	61	1.2	986 to 1320

TABLE 5

Length of retardation period and calcification of the aorta

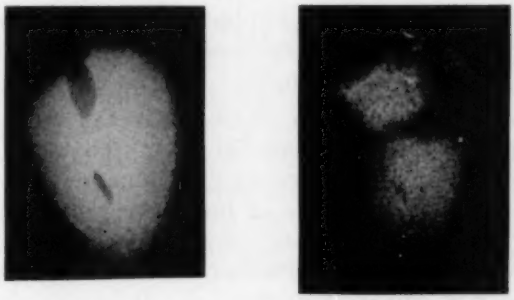
NUMBER OF RATS IN GROUP	LENGTH OF RETARDATION PERIOD	INCIDENCE OF CALCIFICATION OF AORTA	AVERAGE EXTENT OF CALCIFICATION OF AORTA	RANGE OF AGES AT DEATH
	days	%	%	*days*
10 controls	0	20	0.2	713 to 965
7 retardeds	300	29	0.4	711 to 1183
9 retardeds	500	44	1.0	721 to 1103
8 retardeds	700	62	1.4	772 to 1320
8 retardeds	1000 (or less)	100	1.9	815 to 1320

extent of the calcification in the hearts of the rats examined is much less, on the average, than that shown in the kidneys and aortas. A small calcification in the form of a ring near the base of the heart is frequently seen in both controls and retardeds. An example of this may be seen in the upper part of the heart shown in figure 4 a. This is the heart of one of the controls. If we exclude this ring we may say that the calcification in the hearts is more extensive and more frequent in the hearts of the retardeds. Figure 4 b shows the

heart of a retarded rat of the 1000-day group and a typical calcification in the auricles. This rat died at the age of 1004 days. Our x-ray data on the hearts are not sufficiently good to make comparisons of the kind we have made in the cases of aortas and kidneys.

GENERAL PATHOLOGY OF NORMAL AND RETARDED RATS

Limited observations indicate that our stock colony of rats suffers from a high incidence of lung disease when about 1 year of age. There is no way of detecting this ordinarily,



a b

Fig. 4 Slight calcification near the base of the hearts of control rat (a) and retarded rat (b).

however, except by dissection of the animals since they appear in fine health. From the few observations published elsewhere and from our dissection of rats from other sources, it seems that this is general. Nevertheless, it is a matter for serious consideration since it may be a complicating factor of marked importance when the life span is being studied.

If lung disease is important in terminating the life of rats considered normal, its influence must be much less in retarded animals. In the present study a high incidence has been found in both normal and retarded rats at the time of death. As a whole the condition of the lungs in retarded rats at extreme ages has been no different than that found in normal animals

that lived for a much shorter period. In rats retarded for 15 weeks Jackson ('37) found a lower incidence of both middle ear disease and lung disorder among retarded animals. The relation of rickets to rapid growth has long been recognized but the possibility of a similar relationship between diseases of bacterial origin has seemed remote. The growth rate may have a much more profound influence, however, than has been conceived.

The development of tumors in the course of the present study has been given some attention. In the groups permitted to grow normally seven out of fourteen females and two of the seventeen males died with tumors. Some of these tumors weighed nearly as much as the remainder of the animal.

Among the retarded animals only two tumors developed in the course of retardation. Both of these cases were among those retarded for more than 300 days. After retarded animals were allowed to mature, only five tumors were observed in twenty-five cases. Three of these five were in the group of nine rats allowed to mature at 300 days. This evidence is very limited but may indicate that retardation also decreases the number of tumors that develop. It is difficult to know whether the failure of these diseases to develop permits the retarded animals to live longer or whether the retardation modifies the composition of the body so that it is less subject to diseases.

The condition of the kidneys at the time of death varied widely among individuals in respects other than calcification. About a fourth of the control groups and those retarded for the extreme periods of 700 and 1000 days exhibited marked injury. This incidence of diseased kidneys seemed somewhat higher among those retarded for only 300 and 500 days.

SUMMARY

Rats that had been retarded for periods of 300 to 1000 days were used for studying the chemical and pathological changes that accompany aging. The calcium of the eyes was found

to vary from 34 to 1078 mg.% on a dry basis. Most of this calcium is deposited in the eyes after an age of about 900 days. The calcium of the eyes is not correlated with either changes in the bones or with the calcification of other tissues. The teeth of the rats were studied by means of the x-ray. Even at very advanced ages a few animals had intact teeth. About a fourth of all the teeth examined were carious or missing. Females exhibited more damage to their teeth than males although these females never reproduced. A number of cases with bad infections extending into the jaw bones were found. The calcification of the costal cartilages was followed by means of the x-ray. This calcification was much more marked in normal animals than in those of the same age that had been retarded in growth. X-ray photographs of calcified kidneys, hearts and aortas are included. The calcification of the aorta is found to have a much higher incidence among retarded animals than among normals. Indirect evidence indicates that retarded animals are less susceptible to lung disease when a year of age than the normals. Limited evidence also indicates a lower incidence of tumors in retarded animals.

LITERATURE CITED

ELLIS, G. H. 1938 The microdetermination of calcium. J. Ind. and Eng. Chem. (Anal. Ed.), vol. 10, p. 112.

HUMMEL, K. P., AND L. L. BARNES 1938 Calcification of the aorta, heart and kidneys of the albino rat. Am. J. Path., vol. 14, p. 121.

JACKSON, C. M. 1937 Recovery of rats upon refeeding after prolonged suppression of growth by underfeeding. Anat. Rec., vol. 68, p. 371.

MCCAY, C. M. 1939 Problems of Aging. (Edited by Cowdry, E. V.) Williams and Wilkins Co., Baltimore. Chap. 21.

BONE DEVELOPMENT IN NORMAL AND RACHITIC RATS [1]

WALTER C. RUSSELL, M. WIGHT TAYLOR AND MARION T. DUNCAN
Department of Agricultural Biochemistry, New Jersey Agricultural Experiment Station and Rutgers University, New Brunswick

THREE FIGURES

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The extensive use of the white rat for the assay of vitamin D warrants attention to the progressive changes in bone structure that take place during the development of the rachitic condition and during normal growth. Therefore, the object of the present investigation was to obtain a picture of the development of the radii of rats from 3 to 54 days of age on a stock colony ration, and of rats taken from the stock colony group and fed a rachitogenic ration from 24 to 54 days of age. Of the latter period, the portion from 24 to 47 days is that ordinarily used in this laboratory for the depletion of rats for use in vitamin D assays (U. S. P. XI, 1937, Rev.). The stock colony group will be referred to as the normal group.

EXPERIMENTAL PROCEDURE

The stock colony ration was one which has been in use in our colony for 10 years and consists of 60% ground whole wheat, 30% dried whole milk and 10% meat scrap (55% protein), with sodium chloride added to the extent of 2% of the weight of the wheat (Russell, '32). The rachitogenic ration was the well-known Steenbock 2965 (Steenbock and Black, '25).

[1] Journal series paper of the New Jersey Agricultural Experiment Station, department of agricultural biochemistry. Presented before the American Society of Biological Chemists at Memphis, Tennessee, April, 1937.

Radii were collected from two series of animals. The first series covered the period from January to June, 1936, and the number of animals in a litter varied from six to ten. The animals of a litter, nursed by mothers on the stock colony ration, were killed at 3-day intervals from 3 to 24 days of age, inclusive. Whole litters, weaned at 24 days of age, were continued on the stock colony ration or placed on the rachitogenic ration. The members of a litter were killed at 3-day intervals, from 27 to 54 days of age, inclusive. At the completion of the series each 3-day group consisted of five animals.

In order to check the results of the first series with animals selected at a different season of the year, a second collection of radii was made during November and December, 1936. The selection of animals was essentially the same as in the first series except that litters were reduced to eight members each between the third and sixth days and no litters of less than eight were used. Hence one animal from each of five litters was available for each age group for the first 24 days. For the period from 27 to 54 days of age a sufficient number of litters of eight rats each was divided between the two rations so that litter mates on each ration could be killed at intervals throughout the period and provide groups of five animals each at 3-day intervals.

RESULTS AND DISCUSSION

The radii were split, stained with silver nitrate and photographed (Taylor, Klein and Russell, '38). In order to conserve space, a typical bone has been selected from each group of the first series and the bones, arranged in chronological order, are shown in figure 1. At 3 days of age calcification had not appeared in the head of any of the radii, at 6 days of age it was observed in some cases but at 9 days it was present in all members of the group. In the normal animals there was a progressive narrowing of the epiphyseal cartilage with increase in age but an immediate and rapid widening of the cartilage occurred as soon as animals were placed on the

rachitogenic ration. At about 30 days of age, after 6 days on the rachitogenic ration, the bones of the animals of this group began to differ in appearance from those of the normal. The diameter of the shaft tended to remain constant, whereas the head of the bone continued to enlarge. In the normal bones both the head and the shaft continued to grow. This difference in shaft growth gives the heads of the bones of

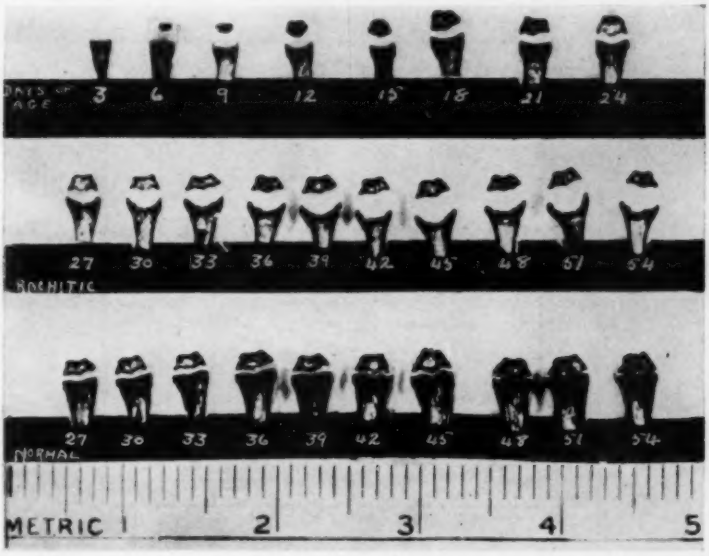

Figure 1

the rachitic series the appearance of having greater width than those of the normal, but measurement shows that the head diameter is essentially the same in the two types of bones.

The growth curves (fig. 2) reveal the marked slowing of growth which took place when the animals were placed on the rachitogenic ration. The gain in weight of animals of this colony during the depletion period (24 to 47 days of age for the preparation of animals for vitamin D assay) varies, and the gain made by the animals of this experiment, about

10 gm., was lower than the 15 to 20 gm. gain frequently observed. Even when the best growth was shown during the depletion period it was markedly less than during the same age period on the colony ration. This sharp change in growth rate indicates that the rachitogenic ration is deficient in certain factors essential for growth and that the storage of these factors is very small. The apparent retardation of the growth rate, immediately after 24 days of age, as shown by the

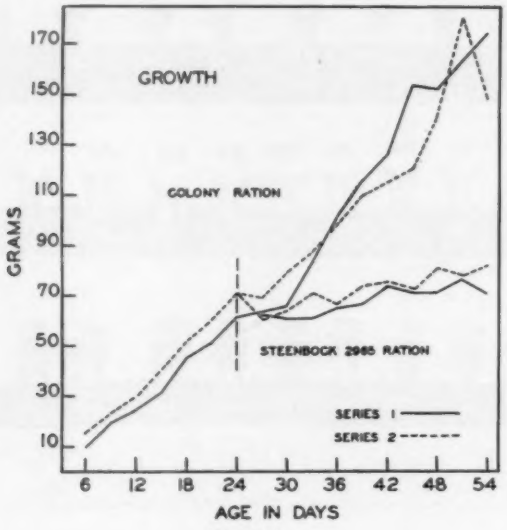

Figure 2

curves for the stock colony animals was probably due to the fact that during the first 24 days litters were progressively reduced so that the remaining rats had a better opportunity for nursing and, therefore, gained more rapidly, whereas animals from whole litters weaned at 24 days were used in obtaining the points on the growth curves for 27 days of age and subsequent ages.

The width of the epiphyseal cartilage has been used as an index of calcification. To make this measurement, the bone

photographs were enlarged so that the diameter of the photograph actually used for the measurement was ten times that of the bone itself. Width values are an average of measurements made at each side and at the center of the cartilage.

Variation of the width of the epiphyseal cartilage with age is shown in figure 3. The width of the cartilage decreased

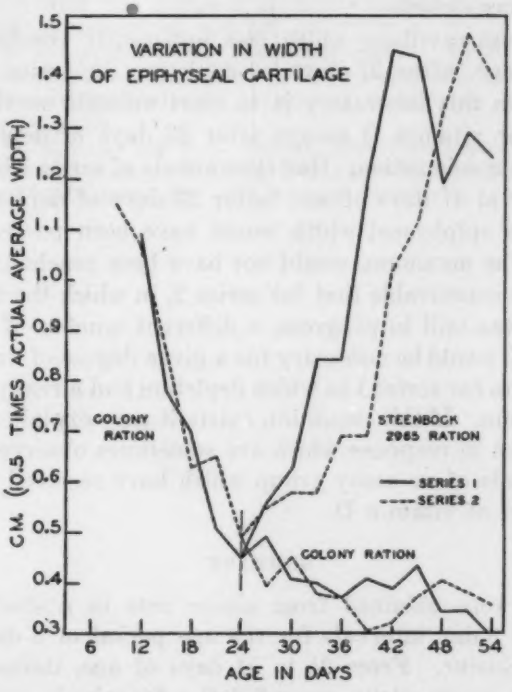

Figure 3

rapidly during the first 24 days of life. Beyond this period the decrease continued in the case of the animals placed on the colony ration but at a slower rate. Immediately after the animals were placed on the rachitogenic ration a rapid widening of the epiphyseal cartilage ensued. In series 1 a maximum width was reached at 42 days of age, which is 18 days

after the start of the depletion period. After the peak was reached, there were fluctuations in width values until the end of the experiment at 54 days of age but no further increase. This observation is in agreement with the experience of this laboratory that when rickets resistance occurs the continuance of animals beyond the usual depletion period does not induce more severe rickets.

Maximum cartilage width was apparently reached at 51 days of age, after 27 days of depletion, in series 2. The practice in this laboratory is to start animals on the assay period for vitamin D assays after 23 days of depletion on the rachitogenic ration. Had the animals of series 1 been used for assay at 47 days of age (after 23 days of depletion) the maximum epiphyseal width would have been passed but in series 2 the maximum would not have been reached. Therefore, it is conceivable that for series 2, in which the depletion process was still in progress, a different number of units of vitamin D would be necessary for a given degree of line calcification than for series 1 in which depletion had already reached a maximum. If this condition exists it may explain the wide differences in response which are sometimes observed among the animals of an assay group which have received identical quantities of vitamin D.

SUMMARY

Radii were obtained from albino rats in a stock colony ration at 3-day intervals for the age period of 3 days to 54 days, inclusive. From 24 to 54 days of age, inclusive, rats from the stock colony were fed the Steenbock rachitogenic ration 2965 and the radii taken at 3-day intervals.

The split, stained radii were photographed and the average width of the epiphyseal cartilage measured. This value for the stock colony animals decreased continuously and tended to become constant after 36 days of age. The narrowing of the epiphyseal cartilage ceased within 3 days after the animals were placed on the rachitogenic ration and a widening process

began which continued for 18 to 27 days (42 to 51 days of age) after which there was no further widening of the cartilage.

There was a marked retardation of the growth rate immediately after the animals were placed on the rachitogenic ration.

LITERATURE CITED

RUSSELL, W. C. 1932 The addition of raw beef or meat scrap to a wheat milk diet. J. Nutrition, vol. 5, p. 347.

STEENBOCK, H., AND A. BLACK 1925 Fat-soluble vitamins. XXIII. The induction of growth-promoting and calcifying properties in fats and their unsaponifiable constituents by exposure to light. J. Biol. Chem., vol. 64, p. 274.

TAYLOR, M. W., D. KLEIN AND W. C. RUSSELL 1938 Photographing line tests in vitamin D assays. Ind. Eng. Chem. (Anal. Ed.), vol. 10, p. 26.

UNITED STATES PHARMACOPEIA XI 1937 Revision. Philadelphia.

FACTORS AFFECTING MANGANESE UTILIZATION IN THE CHICKEN

H. S. WILGUS, JR., AND A. R. PATTON

Poultry Section, Colorado Agricultural Experiment Station, Fort Collins, Colorado

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Numerous workers have found that high levels of calcium and phosphorus in the diet of chickens increase the incidence of perosis. Repeated observations on the aggravation of perosis with sodium phosphate and steamed bone meal as well as statistical studies (Milby, '33; Hammond, '36), have placed the responsibility for this effect upon phosphorus. However, other evidence indicates that this causative effect is not due to phosphorus alone. A review of the literature has shown that phosphorus generally aggravates perosis only when a high level of calcium is also in the diet. Hunter ('31), Payne ('32), Wilgus ('37 a) and their co-workers have found that perosis can be aggravated by a high-calcium diet. On the other hand, perosis is not aggravated by a low-calcium high-phosphorus diet (Schaible et al., '33; Milby, '34; Clifcorn et al., '38).

Feeding large amounts of calcium and phosphorus induces perosis by increasing the requirement for manganese (Caskey and Norris, '38; Schaible et al., '38; Wiese et al., '38 a). Since there is no pronounced change in blood calcium and phosphorus in perosis (Hall and King, '31; Payne et al., '32; Herner and Robinson, '32; Holmes et al., '33; Heller and Penquite, '37; Wilcke, '33; Wiese et al., '38 b), and since manganese injected intraperitoneally or subcutaneously is effective in much smaller quantities than that given in the diet (Lyons, '38; Schaible, '38; Caskey, '38; Weise, '38 b and their co-workers), it seems probable that the effect of excess

calcium and phosphorus takes place in the digestive tract instead of within the body proper. Schaible ('35) has found that calcium phosphate tends to dissolve in the upper digestive tract and to reprecipitate in the lower intestines. We believed this precipitation might result in a removal of soluble manganese, thus causing perosis by creating a virtual manganese deficiency. Proceeding on this hypothesis, further evidence that phosphorus alone is not causative has been obtained and the mechanism by which calcium and phosphorus

TABLE 1

Basal diets used in growth trials

INGREDIENTS	EXPERIMENT NUMBER		
	1	2	3
	%	%	%
Ground yellow corn	69.3	61.2	51.2
Dried buttermilk	10.0	10.0	..
Dried skim milk	..	..	20.0
Sardine meal	15.0	15.0	..
Soybean oil meal	..	..	20.0
Alfalfa leaf meal	5.0	5.0	..
Sodium chloride iodized	0.5	0.5	1.0
Reenforced fish oil (400 D)	0.2	0.3	0.3
Pulverized oystershell	..	..	0.5
Steamed bone meal	..	..	2.0
Total	100.0	92.0	95.0
Ground yellow corn and supplement	..	8.0	5.0

in the diet increase the incidence of perosis has been investigated.

GROWTH TRIAL

Observations on the effect of phosphorus from different sources on the incidence of perosis constitutes the first part of this report. A series of three growth trials was run with White Plymouth Rock chicks. The experimental procedure was essentially the same as that previously used (Wilgus et al., '37 a). The basal diets used in each of the three trials are given in table 1. The supplements added to the diets and the analyses of the final diets are given in table 2. In experiments 2 and 3, the feed containing phosphoric acid was stored

TABLE 2

Growth trials

PEN NO.	SUPPLEMENT	EXPERIMENT 1					EXPERIMENT 2					EXPERIMENT 3					
		Ca	P	Mn	Weight at 6 weeks	Perosis	Ca	P	Mn	Weight at 6 weeks	Perosis	Ca	P	Mn	Weight at 6 weeks	Perosis	Mn per kilogram bone [1]
		%	%	*ppm*	*gm.*	*Degree*	%	%	*ppm*	*gm.*	*Degree*	%	%	*ppm*	*gm.*	*Degree*	*mg.*
1.	None	0.74	0.70	10	565	10	1.21	0.85	10	299	41	1.27	0.82	13	488	16	2.8
2.	St. Bone Meal	..	..	..	..	..	2.13	1.27	10	257	48	2.19	1.24	13	474	36	2.6
3.	$MnSO_4 \cdot 2H_2O$	..	..	..	..	..	2.13	1.27	60	333	0	1.27	0.82	63	561	0	5.0
4.	H_3PO_4	0.74	1.02	10	567	1	1.21	1.30	10	266	33	1.27	1.27	13	499	10	3.2
5.	$CaCO_3$	..	..	..	..	..	2.11	0.85	10	303	45	2.17	0.82	13	466	13	3.2
6.	Na_2CO_3	0.74	0.70	10	543	14	..	..	..	..	..	1.27	0.82	13	549	9	3.2
7.	$H_3PO_4 + CaCO_3$	..	..	..	..	..	2.11	1.27	10	266	53	2.17	1.27	13	518	25	3.1
8.	$H_3PO_4 + Na_2CO_3$	0.74	1.02	10	574	2	..	..	..	..	..	1.27	1.27	13	510	3	3.6
9.	$NaH_2PO_4 \cdot H_2O$ $FeC_6H_5O_7 \cdot 3H_2O$	0.74	1.04	10	499	18	..	..	..	..	..	..	..	..	..	..	..
10.	0.17%, dry	0.74	0.70	10	542	25	..	..	..	..	..	..	..	..	..	..	..
11.	0.17%, wet	0.74	0.70	10	526	32	..	..	..	..	..	..	..	..	..	..	..
		15 chicks per pen					12 chicks per pen					20 chicks per pen					

[1] Fat-free dry bone.

in cans lined with cotton sacks and the galvanized feeders and grills were heavily coated with bakelite varnish.

The results of these growth trials are given in table 2. In all three experiments, phosphoric acid added alone to make the total phosphorus between 1.0 and 1.3% in a basal diet containing from 0.74 to 1.27% of calcium not only failed to aggravate perosis but actually appeared to alleviate the symptoms somewhat. The addition of sodium carbonate in experiments 1 and 3 alone, or with phosphoric acid in an amount to approximate monosodium phosphate, had no effect. The C.P. monosodium phosphate used in experiment 1, presumably as a standard of comparison for the production of perosis, failed likewise to be markedly aggravating, although more so than the basal diet alone. This sample of monosodium phosphate depressed growth to quite an extent but did not show any significant impurities on later spectrographic examination.

Raising the calcium content of the diet to about 2.1% with calcium carbonate did not aggravate perosis to the anticipated extent at a phosphorus level of about 0.8%. This might be expected in experiment 2, in which the severity of perosis was already great on the basal diet alone. The ineffectiveness in experiment 3 could be accounted for only if the small amount of manganese found on this salt (8 p.p.m.) was more effective when fed combined with the salt rather than alone. Discrepancies due to manganese contamination have been observed before (Wilgus et al., '37 b; Schaible et al., '38). However, when the calcium and the phosphorus levels were increased simultaneously to about 2.1% and 1.3% respectively, the incidence of perosis was enhanced. In view of the ineffectiveness of phosphorus increment alone, calcium is essential to increase the incidence of perosis.

Ferric citrate was added to the diet in experiment 1 to clarify previous conflicting results (Wilgus et al., '37 b) obtained on this material. These data definitely showed that this salt was causative.

Manganese was determined in the combined tibiae, femora and metatarsi of the left legs of five birds from each pen in experiment 3. The data show a very low concentration of manganese in all pens, although the manganese supplementation slightly increased the concentration of that element in the bones. However, this amount while somewhat higher than that reported by Gallup and Norris ('38) and by Schaible et al. ('38), is so small as to suggest that manganese is not a component part of bone structure.

IN VITRO TRIAL

Since the results of the growth trial supported the hypothesis advanced, further experiments were carried on in the laboratory. A solution of calcium nitrate was added to a solution of phosphoric acid and manganous sulfate. Concentrations of calcium, phosphate and manganous ions were similar to those found in the diets used in pens 1 and 7 of experiment 3. At a slightly acid reaction to simulate the pH of the lower digestive tract, the system was heated to boiling, cooled, the calcium phosphate precipitate removed by filtration and the precipitate washed. Manganese determinations showed the removal of all manganese from solution by the calcium-phosphate precipitate.

A similar experiment was performed with concentrations of iron and manganese similar to those used in pens 10 and 11 of experiment 1, except that ferric chloride was used. The solutions were boiled with the addition of a few cubic centimeters of n/10 NaOH to facilitate the flocculation of ferric hydroxide. On filtering and washing, the manganese was recovered in the precipitate.

These two series of experiments supported the hypothesis that the availability of manganese in the digestive tract of the chicken is decreased by an excess of calcium and phosphorus or by excess iron through adsorption or combination with the insoluble salts formed. As a final test, a feeding trial was conducted.

IN VIVO TRIAL

Three groups of four White Leghorn pullets approximately 18 weeks old and of about the same weight were placed on the following diets:

Pen 1—Perosis basal diet (experiment 3), 0.96% calcium, 0.80% phosphorus.

Pen 2—Basal diet plus 50 ppm. manganese; 0.96% calcium, 0.80% phosphorus.

Pen 3—Basal diet plus 50 ppm. manganese plus 7% steamed bone meal; 2.92% calcium, 1.65% phosphorus.

At the end of 10 days, the birds were sacrificed and contents of the crop, proventriculus and gizzard, duodenum,

TABLE 3

Solubility of manganese in the digestive tract

	TOTAL MANGANESE			DIALYZABLE MANGANESE		
	1 Basal	2 Mn	3 Mn + St.B.M	1 Basal	2 Mn	3 Mn + St.B.M
	mcg	*mcg*	*mcg*	%	%	%
1. Feed	346	1554	1090	29	38	22
2. Crop	141	1250	917	40	38	26
3. Proventriculus and gizzard	96	137	277	74	71	53
4. Duodenum	63	40	38	84	63	80
5. Small intestine						
a. Upper half	188	1086	471	61	80	32
b. Lower hlaf	229	1002	872	41	38	12
a + b	417	2088	1343	50	60	19
6. Colon	20	83	102	100	100	80
7. Caeca	45	191	128	78	0	12
Total 4 to 7 inclusive	545	2402	1611	58	57	24
8. Excreta	137	5156	4173	5	15	3

upper half of the small intestine, lower half of the small intestine, colon and caeca were removed separately. Samples of each diet and of excreta were also used. The pH of each sample was determined immediately on removal. The samples from the individuals in each pen were then combined in each of the seven fractions and poured into cellophane tubes. About 18 hours elapsed between these two steps; hence, the pH on the combined fractions were redetermined just previ-

ous to transfer. The samples were then dialyzed against distilled water for 3 days, with six changes of water.

The combined diffusates for each fraction were evaporated to dryness with about 10 cc. of HNO_3 and ashed. Total manganese was then determined by the periodate method.

The data thus obtained are summarized in table 3. They show that the presence of excess calcium and phosphorus in the diet definitely depressed the solubility of manganese in the digestive tract. This appeared to be true even in the feed,

TABLE 4

pH of feed, digestive material and excreta

MATERIAL	FRESH MATERIAL			18 HOURS LATER		
	1 Basal	2 Mn	3 Mn + St.B.M	1 Basal	2 Mn	3 Mn + St.B.M
1. Feed	6.5	6.4	6.5	..	..	..
2. Crop	4.5	5.2	4.8	4.4	4.4	4.2
3. Proventriculus and gizzard	3.5	3.3	3.3	4.0	3.8	4.1
4. Duodenum	6.0	6.2	6.0	5.3	5.6	5.7
5. Small intestine						
a. Upper half	6.1	6.3	6.3	5.6	5.5	5.8
b. Lower half	7.2	7.3	7.4	5.5	5.6	6.0
6. Colon	6.4	6.9	6.9	5.3	5.2	5.8
7. Caeca	5.5	6.5	6.1	5.4	6.6	6.5
8. Excreta	..	..	..	5.5	5.2	5.2

in the crop and in the proventriculus and gizzard. The figures are particularly impressive in the lower half of the small intestine and in the excreta. The small amount of material and of manganese in the duodenum, colon and caeca render data for these portions of less significance.

As shown in table 4, the pH of the materials decreased with standing at room temperature. Since this would tend to increase the availability of manganese, it seems likely that even more striking results would have been obtained if this shift in pH could have been avoided.

DISCUSSION

These findings strongly indicate that the perosis-aggravating action of such supplements as steamed bone meal and calcium phosphate is due to the removal of manganese from solution by the excess insoluble calcium phosphate in the intestines. The same explanation may hold for the increase in perosis following the addition of calcium salts without providing additional phosphorus, since the excess of calcium in the digestive tract would favor the formation of insoluble calcium phosphate. Likewise, when phosphorus supplementation is made to high calcium diets, as is usually the case, the formation of insoluble phosphate would be facilitated by the increased amount of both phosphorus and calcium in the digestive tract.

A calculation of the amounts of manganese injected by Lyons ('38), Schaible ('38), Gallup ('38) and Wiese ('38 b) and their co-workers into approximate concentration in the diet shows that from 6 to 10 p.p.m. was thus injected. The minimum protective levels of manganese required to be added to the basal diets for comparable protection was shown to be from 25 to 30 p.p.m. by Insko ('38), Gallup ('38 a) and Schaible ('38) and their co-workers. This indicates that not more than 20 to 40% of the manganese is available when added to the diet in these amounts. These percentages, calculated on the basis of assumed feed consumption, are comparable to those given in this report for the small intestines.

The stimulation of perosis by a small addition of ferric citrate to the diet may be explained by the formation of ferric chloride in gastric digestion and by hydrolysis to insoluble ferric hydroxide in the less acid intestines. This reaction would tend to remove the manganese in the manner demonstrated in vitro.

It is possible that the action of calcium phosphate in the digestive tract may prove to be the same mechanism that increases the iodine requirement (Thompson, '36) and inter-

feres with iron assimilation (Kletzien, '38) through the feeding of excess calcium.

The fact that manganese occurs in such a limited concentration in fat-free-dry bone is in agreement with the report of Gallup and Norris ('38) and of Schaible et al. ('38) and would seem to indicate that this element is not a component part of bone structure. The evidence brought forth by Wiese et al. ('38 b) that bone phosphatase is subnormal in active perosis and the report that manganese catalyzes phosphatase activity (Thannhauser et al., '37) might lead one to conclude that the effect of manganese is the result of its catalytic effect on phosphatase. The low serum phosphoric ester reported by these workers may be a result of low serum phosphatase, as this enzyme acts reversibly.

However, the primary effect of manganese in bone growth may be found to relate more closely to bone cartilage than to bone salt deposition. The well-known fact that the ash, calcium and phosphorus content of bone is normal in perosis and the incidence of chondrodystrophy in chick embryos from hens on a low-manganese diet (Lyons and Insko, '37) support this possibility.

SUMMARY

The finding that phosphorus supplementation to a perosis-producing diet did not aggravate perosis indicates that phosphorus per se is not a causative factor. Excess calcium in the diet is apparently essential for the stimulation of perosis.

It has been shown that insoluble calcium phosphate can remove manganous ions from solution. The decrease in diffusible manganese in the intestinal contents of chicks which was observed after feeding steamed bone meal is apparently due to this action of calcium phosphate.

Ferric hydroxide also removed manganese from solution. Ferric citrate in the diet increased the severity of perosis.

LITERATURE CITED

CASKEY, C. D., AND L. C. NORRIS 1938 Further studies on the role of manganese in poultry nutrition. Poultry Sci., vol. 17, p. 433.

CLIFCORN, L. E., C. A. ELVEHJEM AND E. B. HART 1938 The development of a ration for the study of perosis in chicks. Poultry Sci., vol. 17, p. 28.

GALLUP, W. D., AND L. C. NORRIS 1937 Studies of the perosis-preventive properties of manganese. J. Biol. Chem., vol. 117, p. xxxvi.

———— 1938 The essentialness of manganese for the normal development of bone. Science, vol. 87, no. 2245, p. 18.

HALL, G. E., AND E. J. KING 1931 Calcium and phosphorus metabolism in the chicken. II. Range paralysis. Poultry Sci., vol. 10, p. 259.

HAMMOND, J. C. 1936 Inorganic phosphorus and perosis. Poultry Sci., vol. 15, p. 260.

HELLER, V. G., AND R. PENQUITE 1937 Factors producing and preventing perosis in chickens. Poultry Sci., vol. 16, p. 243.

HERNER, M. C., AND A. D. ROBINSON 1932 A study of leg bone deformities in growing chicks. Poultry Sci., vol. 11, p. 283.

HOLMES, A. D., M. G. PIGOTT AND W. B. MOORE 1933 Mineral content of tibia from chicks with slipped tendon. Poultry Sci., vol. 12, p. 356.

HUNTER, J. E., R. A. DUTCHER AND H. C. KNANDEL 1931 Further studies on the production of experimental 'slipped tendons' or 'hock diseases' in chicks. Poultry Sci., vol. 10, p. 392.

INSKO, W. M. JR., M. LYONS AND J. H. MARTIN 1938 The quantitative requirement of the growing chick for manganese. J. Nutrition, vol. 15, p. 621.

KLETZIEN, S. W. 1938 The influence of calcium and phosphorus on iron assimilation. J. Nutrition, vol. 15 (Suppl.), p. 16.

LYONS, M., AND W. M. INSKO, JR. 1937 Chondrodystrophy in the chick embryo produced by manganese deficiency in the diet of the hen: Kentucky Agr. Exp. Sta. Bull. no. 371.

LYONS, M., W. M. INSKO, JR. AND J. H. MARTIN 1938 The effect of intraperitoneal injections of manganese, zinc, aluminum and iron salts on the occurrence of slipped tendon in chicks. Poultry Sci., vol. 17, p. 12.

MILBY, T. T. 1933 A statistical analysis of some experiments on slipped tendon. Poultry Sci., vol. 12, p. 352.

———— 1934 Factors influencing a malformation of the leg bones of growing chickens. Iowa Agr. Exp. Sta. Res. Bull. no. 172.

PAYNE, L. F., J. S. HUGHES AND H. F. LIENHARDT 1932 The etiological factors involved in the malformation of bones in young chickens. Poultry Sci., vol. 11, p. 158.

SCHAIBLE, P. J., J. M. MOORE AND R. A. CONOLLY 1933 Factors influencing the incidence of perosis in barred rock chicks. Poultry Sci., vol. 12, p. 325.

SCHAIBLE, P. J., S. L. BANDEMER AND J. M. MOORE 1935 Some aspects of high mineral metabolism in chicks. Poultry Science, vol. 14, p. 303.

SCHAIBLE, P. J., S. L. BANDEMER AND J. A. DAVIDSON 1938 The manganese content of feedstuffs and its relation to poultry nutrition. Mich. St. Col. Agr. Exp. Sta. Tech. Bull. no. 159.

THANNHAUSER, S. J., M. REICHEL AND J. F. GRATTON 1937 Studies on serum phosphatase activity. J. Biol. Chem., vol. 121, p. 697.

THOMPSON, J. 1936 Influence of the calcium intake on the blood iodine level. Endocrinol., vol. 20, p. 809.

WIESE, A. C., C. A. ELVEHJEM AND E. B. HART 1938 a Studies of the prevention of perosis in the chick. Poultry Sci., vol. 18, p. 33.

WIESE, A. C., B. C. JOHNSON, C. A. ELVEHJEM AND E. B. HART 1938 b Phosphorus metabolism of chicks afflicted with perosis. Science, vol. 88, no. 2286, p. 383.

WILCKE, H. L. Thesis, cited by T. T. Milby, 1934, loc. cit.

WILGUS, H. S. JR., L. C. NORRIS AND G. F. HEUSER 1937 a The effect of various calcium and phosphorus salts on the severity of perosis. Poultry Sci., vol. 16, p. 232.

———— 1937 b The role of manganese and certain other trace elements in the prevention of perosis. J. Nutrition, vol. 14, p. 155.

THE UTILIZATION OF ENERGY PRODUCING NUTRIMENT AND PROTEIN AS AFFECTED BY THE LEVEL OF THE INTAKE OF BEEF MUSCLE PROTEIN [1]

E. B. FORBES, ALEX BLACK, EDWARD J. THACKER AND R. W. SWIFT
Institute of Animal Nutrition, The Pennsylvania State College, State College

FOUR FIGURES

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In 1935 and 1937 papers were published from this laboratory (Forbes, Swift, Black and Kahlenberg, '35; and Forbes, Voris, Bratzler and Wainio, '37) setting forth results of experiments on the plane of protein intake as affecting the utilization of food energy and protein, the main nitrogenous component of the diets having been casein.

These experiments were in excellent agreement in revealing moderate decrease in heat production with increase in the protein content of equicaloric diets containing 10, 15, 20, 25, 30, 35 and 45% of protein. However, certain questions remained as to whether the facts characterizing casein in this connection are true of protein in general, and also as to the influence of the plane of food intake in the same relations.

The experiments to be discussed, therefore, were designed to contribute to the answer of these two questions.

The procedure was the same as in the earlier studies of the same general subject; that is, the body gains of energy and of protein were determined by difference between values for the body components of a control group representing the experimental subjects at the beginning of the investigation and

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parallel values for subjects at the end of the 70-day experiment; and the heat production was measured as the gross energy of the food minus the energy of the excreta and of the body gain for the entire period.

As in the earlier experiments the rats were fed with quadruplet food control. The protein contents of the diets were 10, 25, 35 and 45%, respectively, each rat of a quadruplet receiving a different intake of protein, but the same intake of energy.

The twelve quadruplets of rats serving as experimental subjects were divided into two groups of six quadruplets each, one of which was held to approximately the same food intake as that which prevailed in the earlier experiments with casein, while the other was permitted to consume food at a much higher rate, determined for each quadruplet by the ad libitum consumption of the poorest eater in each quadruplet. The average consumption of food energy in this latter group of six quadruplets was 44.7% higher than the average in the earlier experiments.

The composition of the diets as given in table 1 requires no comment.

In table 2 it is shown that the higher plane of food intake led to the larger body gains at each percentage of protein—which is virtually inevitable.

The relative efficiency of the diets to produce gain in body weight is indicated in figures 1 and 2. These graphs repreent gross body weight.

The nitrogen and the fat gained, and the quantitative relation of the one to the other, are determined as resultants of a complication of factors, to wit—the amounts and proportions of protein and non-protein nutriment 1) in the diets, 2) required for maintenance, and 3) available for body increase.

At the lower plane of food intake the comparatively low values for both nitrogen and fat gained only suggest intake less than the optimum. The rapid decrease in fat gained with increasing order of protein contents of the diets implies di-

TABLE 1

Composition of diets

	10% PROTEIN RATION	25% PROTEIN RATION	35% PROTEIN RATION	45% PROTEIN RATION
	%	%	%	%
Cellu flour	4.00	4.00	4.00	4.00
O. and M. salt mixture	4.00	4.00	4.00	4.00
NaCl	1.00	1.00	1.00	1.00
Butterfat	1.00	1.00	1.00	1.00
Yeast [1]	6.00	6.00	6.00	6.00
Dextrin	62.34	49.29	40.59	31.89
Beef protein	8.19	26.55	38.80	51.04
Crisco	13.47	8.16	4.61	1.07
	100.00	100.00	100.00	100.00

[1] The yeast mixture contained 5 parts of brewer's yeast to 1 part of irradiated yeast.

Note: Carotene, in oil, was added to each diet in the proportion of 0.333 gm. per kilogram. Each diet contained approximately 1.4 U.S.P. units of vitamin A per gram.

TABLE 2

Food eaten and average quantities, character and gross efficiency of gains in weight during 70 days

PER CENT OF PROTEIN IN DIET	INITIAL LIVE WEIGHT	FINAL LIVE WEIGHT	FOOD EATEN (DRY MATTER)	GAIN IN EMPTY BODY WEIGHT	FOOD (DRY MATTER) PER GRAM BODY GAIN	NITROGEN OF BODY GAIN	FAT GAINED	FAT GAINED PER GRAM N GAINED
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Lower food intake								
10	48	123	430	76	5.7	2.5	12.60	5.0
25	48	141	430	94	4.6	3.5	8.35	2.4
35	48	139	430	91	4.7	3.4	7.07	2.1
45	48	132	429	83	5.2	3.2	4.31	1.3
Higher food intake								
10	49	173	630	121	5.2	3.7	24.31	6.6
25	50	218	630	167	3.8	5.5	29.61	5.4
35	50	220	630	168	3.8	5.8	22.81	3.9
45	50	207	629	154	4.1	5.2	23.91	4.6

Note: All data in all tables are averages per rat, from groups of six on the same dietary treatment, for 70 days.

minishing non-nitrogenous nutriment; and the associated gain in body nitrogen, from the 10% protein to the 25% protein diet, followed by a decrease in gain in nitrogen from the 25% protein to the 45% protein diet, seems to be due primarily to the fact that the protein was unaccompanied by enough non-protein nutriment to provide for its efficient utilization.

At the higher plane of nutrition the amounts of nitrogen and of fat gained were inevitably much higher than at the lower plane, with the best conditions for gain of nitrogen

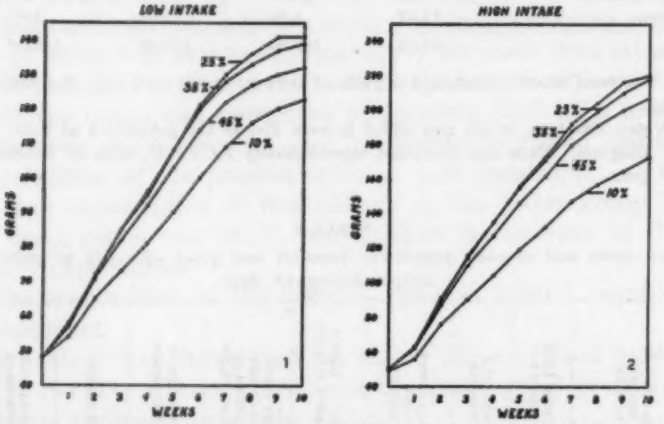

Fig. 1 Shows average gains in body weight of groups of six growing rats as affected by the plane of intake of beef muscle protein in equicaloric diets consumed at a restricted rate.

Fig. 2 Shows average gains in body weight of groups of six growing rats as affected by the plane of intake of beef muscle protein in equicaloric diets consumed at a relatively liberal rate.

with the 35% protein diet and for gain of fat with the 10% protein diet.

It is clear, therefore, that the character of the body increase as to proportion of fat to protein differs in accord with character of the food.

The distribution of the food energy as affected by the plane of intake and by the percentage of protein in the equicaloric diets is shown in table 3 and in figures 3 and 4.

TABLE 3

Distribution of average total food energy[1] as affected by the plane of protein intake

PER CENT OF PROTEIN IN DIET	FOOD ENERGY	FECES	DIGESTED	URINE	METABOLIZED	BODY GAIN	BODY GAIN AS PROTEIN	BODY GAIN AS FAT	HEAT
	Cal.	*Cal.*	*Cal.*	*Cal.*	*Cal.*	*Cal.*	*Cal.*	*Cal.*	*Cal.*
Lower food intake									
10	2138	216	1922	42	1880	210	91	119	1670
25	2138	186	1952	98	1854	201	122	79	1653
35	2138	178	1960	142	1818	187	120	67	1631
45	2138	187	1951	183	1768	156	115	41	1612
Higher food intake									
10	3136	339	2797	58	2739	364	136	228	2375
25	3136	296	2840	142	2698	480	201	279	2218
35	3136	279	2857	209	2648	423	208	215	2225
45	3136	298	2838	261	2577	415	189	226	2162

[1] For 70 days.

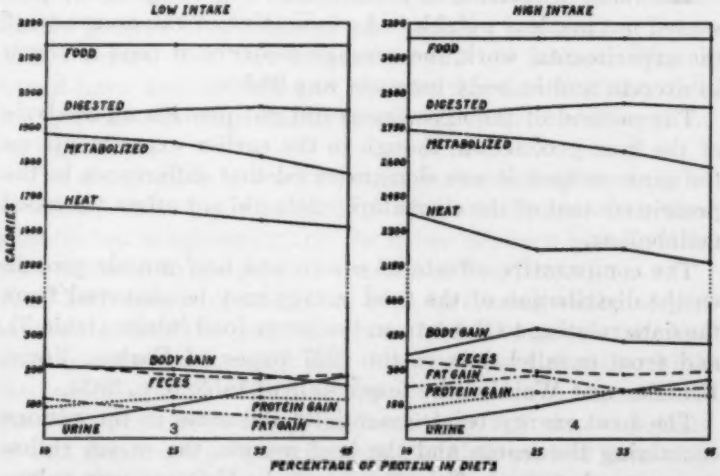

Fig. 3 Illustrates the distribution of food energy by groups of six growing rats as affected by the plane of intake of beef muscle protein in equicaloric diets consumed at a restricted rate.

Fig. 4 Illustrates the distribution of food energy by groups of six growing rats as affected by the plane of intake of beef muscle protein in equicaloric diets consumed at a relatively liberal rate.

The more notable facts in this relation, at the two planes of food intake, were 1) that the digestibility of the nutrients containing the food energy increased slightly from the 10% to the 35% protein diets, and then decreased very slightly between the 35% and the 45% protein diets; 2) that the urine energy increased markedly and regularly from the 10% to the 45% protein diet; 3) that the metabolizability of the diets diminished at a nearly regular rate from the 10% to the 45% protein diet; 4) that the heat production diminished slightly from the 10% to the 45% protein diet, at a rate less than the accompanying decrease in metabolizable energy at the lower plane of nutrition, but greater than the decrease in metabolizable energy at the higher plane of nutrition; 5) that the body gain diminished materially at the lower plane of intake and increased slightly (with some irregularity) at the higher plane of food intake, from the 10% to the 45% protein diet.

The facts in relation to protein gain and fat gain are presented but are less notable. As indicative of the accuracy of the experimental work, the average recovery of food nitrogen in excreta and in body increase was 98.5%.

The method of the experiment did not provide an analysis of the heat production, though in the earlier experiments on the same subject it was demonstrated that differences in the protein content of the equicaloric diets did not affect the basal metabolism.

The comparative effects of casein and beef muscle protein on the distribution of the food energy may be observed from the data relating to the rats on the lower food intake (table 3), and from parallel data in the 1937 paper of Forbes, Voris, Bratzler and Wainio (the lower half of table 3, p. 293).

The food energy being essentially the same in the rations containing the casein and the beef muscle, the casein ration was somewhat more efficiently digested. Urine energy values were almost identical. Metabolizable energy values, therefore, differed as the digestible energy values differed.

Values for energy of body gain were higher for the casein; as to heat production, the values differed but little—between

1699 to 1576 for the casein ration and 1670 to 1612 for the beef protein ration.

These data, therefore, reveal no evidence whatever of a greater heat stimulating effect of beef muscle protein than of casein for growing rats.

The metabolizability of the food energy is obviously a factor of importance in relation to the heat production, but that this factor alone did not control the heat production is indicated by the facts that at the lower plane of nutrition the metabolizable energy diminished about twice as much as did the heat production, from the 10% and the 45% diets, while at the higher plane of nutrition the heat production diminished more rapidly than did the metabolizable energy, with increase in the protein content of the equicaloric diets.

As in the earlier experiments on the same general subject, metabolizable energy was computed as that of the food minus that of the excreta without correction for the non-metabolizable portion of the energy of the body increase.

The reason that this correction was not made is that it would have had the effect to obscure the values of the diets for growth—an important consideration in the present investigation.

The distribution of food nitrogen, as accounted for in table 4, shows that the digestibility of the nitrogen increased slightly but consistently and the urine nitrogen prominently, with increase in the protein content of the diets.

At both planes of nutrition, with increasing order of protein contents of the diets the grams of food nitrogen retained first increased materially and then decreased slightly; while the percentage of the food nitrogen retained was highest with the 10% protein diet and diminished to less than one-third of this maximum proportion with the 45% protein diet.

That the calories per gram of nitrogen in the urine, as set forth in table 5, invariably decreased in accord with progressively greater protein contents of the diets signifies a decrease in the proportion of nitrogenous compounds of high energy value per gram of nitrogen.

TABLE 4

Distribution of average total food nitrogen as affected by the percentage of protein in the diet

PER CENT OF PROTEIN IN DIET	FOOD NITROGEN	FECES NITROGEN	DIGESTED NITROGEN		URINE NITROGEN		BODY GAIN OF NITROGEN	
	gm.	*gm.*	*gm.*	*%*	*gm.*	*%*	*gm.*	*%*
Lower food intake								
10	7.3	1.0	6.3	86.3	4.0	54.8	2.5	34.2
25	18.2	1.4	16.8	92.3	12.7	69.8	3.5	19.2
35	25.5	1.8	23.7	92.9	19.8	77.6	3.4	13.3
45	32.4	2.1	30.3	93.5	26.4	81.5	3.2	9.9
Higher food intake								
10	10.7	1.6	9.1	85.0	5.3	49.5	3.7	34.6
25	26.7	2.3	24.4	91.4	17.9	67.0	5.5	20.6
35	37.3	2.9	34.4	92.2	28.0	75.1	5.8	15.5
45	47.5	3.4	44.1	92.8	38.0	80.0	5.2	10.9

TABLE 5

Relationship of nitrogen to energy in urine as affected by the proportion of the protein in the diet

PER CENT OF PROTEIN IN DIET	NITROGEN OF URINE	ENERGY OF URINE	ENERGY PER GRAM OF NITROGEN IN URINE
	gm.	*Cal.*	*Cal.*
Lower food intake			
10	4.0	42	10.6
25	12.7	98	7.7
35	19.8	142	7.2
45	26.4	183	6.9
Higher food intake			
10	5.3	58	10.9
25	17.9	142	7.9
35	28.0	209	7.5
45	38.0	261	6.9

There is in the results of this experiment, therefore, no evidence whatever of an increase in heat production associated with progressively greater protein contents of the equicaloric diets; in fact the findings were in unmistakable agreement with all of the previous observations showing that with higher percentages of protein in the diets there is decrease in heat.

In seeking to explain this result the mind naturally turns to the metabolizability of the diets and to the basal metabolism and the voluntary activity.

In regard to the metabolizable energy of the diets, it has been shown that at the 10, 15 and 20% protein levels there is no decrease in metabolizable energy with increasing protein contents of the diets, though the heat production diminishes materially at these levels. Whatever was the influence of the diminishing metabolizability of the diets on the heat production at the higher protein levels, this factor cannot be regarded as the dominant one.

Basal metabolism was not determined in connection with the present experiment, but in the previous studies of the same subject covering the same range of percentages of dietary protein no change in the basal metabolism was observed.

In all probability a special investigation of this aspect of the matter would be required for the complete establishment of the facts, since the decrease in heat with increase in protein was not particularly extensive, and the factoring of this decrease could readily go wrong in any except a thorough investigation of that particular matter. It is, therefore, probably wise to hold in abeyance a decision as to whether the demonstrated decrease in heat production coincident with increasing percentages of dietary protein signifies decrease in basal metabolism, or in voluntary activity, or in both, though the evidence now at hand indicates that the decrease is in the voluntary activity.

Further light will be thrown on the whole problem of the present investigation by experiments now in progress with mature animals, which cannot store much protein, as subjects.

This investigation should reveal the influence on the heat production of protein which is utilized for fat or for energy production.

SUMMARY

The influence of progressively greater protein contents of equicaloric diets was studied by means of feeding, metabolism and body analysis experiments with two groups, twenty-four each, of albino rats as subjects with quadruplet food control, the principal food protein being from beef muscle.

One group was limited in food consumption to the approximate amount eaten in earlier experiments with casein as the principal food protein, and the other was allowed to eat 44.7% more.

The most notable observations relating to the distribution of food energy were: 1) that the metabolizability of the diets diminished at a nearly regular rate from the 10% to the 45% protein diet; 2) that the heat production diminished slightly from the 10% to the 45% protein diet at a rate less than the accompanying decrease in metabolizable energy at the lower plane of nutrition, but greater than the decrease in metabolizable energy at the higher plane of nutrition; 3) that the body gain diminished materially at the lower plane of intake and increased slightly at the higher plane of food intake from the 10% to the 45% protein diet.

The distribution of the food nitrogen and the composition of the body increase, as affected by the composition of the diets, were also observed.

By comparison with earlier work it is shown that beef muscle protein does not have a greater heat stimulating effect than casein for growing rats.

LITERATURE CITED

FORBES, E. B., R. W. SWIFT, ALEX BLACK AND O. J. KAHLENBERG 1935 The utilization of energy producing nutriment and protein as affected by individual nutrient deficiencies. III. The effects of the plane of protein intake. J. Nutrition, vol. 10, pp. 461–479.

FORBES, E. B., LEROY VORIS, J. W. BRATZLER AND WALTER WAINIO 1937 The utilization of energy producing nutriment and protein as affected by the plane of protein intake. J. Nutrition, vol. 15, pp. 285–307.

DYNAMIC EFFECTS AND NET ENERGY VALUES OF PROTEIN, CARBOHYDRATE AND FAT [1]

E. B. FORBES, JOHN W. BRATZLER, EDWARD J. THACKER AND L. F. MARCY

Institute of Animal Nutrition, The Pennsylvania State College, State College

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This investigation, with growing albino rats as subjects, was based on the following fundamental conceptions: 1) that a nutrient is never, under any conditions, metabolized by itself—regardless of whether it is of body origin, as during fast, or is fed by itself to a subject in the postabsorptive state, or is superimposed upon a maintenance diet; 2) that the dynamic effect of a food substance depends on the amount and kind of nutriment with which it is utilized and on the length of time since food was received; and 3) that the conditions under which any nutrient possesses its maximum, normal nutritive value—the logical conditions, therefore, for the measurement of dynamic effects and net energy values—are as in complete and perfect diets or rations (Forbes, '33).

These conceptions may be considered as logical consequences of the ideas of Rubner ('02) who understood that the energy expense of utilization of a mixed diet is less than the sum of such values for the components of the diet, and who also understood the influence of the sparing of body nutrients by food nutrients fed at planes of nutrition below energy equilibrium in relation to the apparent dynamic effect of the food nutrients.

Many followers of Rubner, and later of Lusk, have also subscribed to these ideas from time to time in various relations, but have not followed them consistently in the determination of dynamic effects of nutrients.

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In fact, much the greater part of the literature on this subject is based on observations which the authors of this paper believe to be at least questionable and of indefinite nutritive significance, since they have been made in experiments on subjects at planes of nutrition below maintenance (usually at the so-called basal plane) in which there was, or may have been, a sparing of body nutrients by the food nutrients under investigation.

An appreciation of the facts in this situation led to the conclusion by Forbes, Braman, Kriss et al. ('30) that a heat increment determined at a plane, or between planes, of nutrition below maintenance is less than the true energy expense of food utilization by the amount of energy by which the nutrient fed has spared the katabolism of body substance.

The point of view of the Institute of Animal Nutrition of the Pennsylvania State College with reference to the measurement of specific dynamic effects, which is based on the experimental procedures of Kellner, and of Armsby, as modified since 1922 by Forbes and associates, is set forth in detail in a recent paper by Kriss, Forbes and Miller ('34).

The main features of the procedure advocated are: 1) the measurement of dynamic effects as the difference in the heat production in comparable experimental periods, with the animal established on and thoroughly adjusted to continuing nutritive regimens differing only in the amount of the component of interest, thus permitting heat measurements under such conditions and during such time that values of actual nutritive significance may be obtained; and 2) the use of the status of energy equilibrium as the base from which the heat increment is measured, instead of any condition of fast, as a means of avoiding confusion of the dynamic effect of the food nutrient with the waste heat of utilization of the body nutrients katabolized during experimental periods below the maintenance level.

Obviously the difference between this and the usual point of view of students of the subject is all-important; and, in the absence of correct understanding and general agreement

as to these considerations, the discussion of intimate details of underlying physiology and thermodynamics has very naturally led only to continuing confusion of thought.

But whatever the understanding of the fundamental facts involved in this problem, and whatever the details of experimental procedure employed, the energy expenses of food utilization in general, and the dynamic effects of specific nutrients, appear to be so much affected by so many influences having to do with nutrient combinations, planes of nutrition, and the purposes for which the nutrients are used, that if such values are to possess the significance of constants this must be by virtue of rigorously formalized definition and experimental control. The values, therefore, apply with accuracy only to the special conditions under which they have been determined.

In the investigation to be discussed determinations were made of the dynamic effects and net energy values of protein, carbohydrate and fat, singly and of the three combined, these supplements being superimposed, in all cases, upon an approximately complete, basal, maintenance diet.

It will be understood that in any determination of heat increments the entire supplementing or sparing effect of the combination of the basal with the test nutrients must be referred to the 'test nutrients' since there is no scientific method for apportioning the value of a diet among its constituents—a point of view which becomes obvious and indisputable when it is realized that inorganic salts, which possess no energy value in the usual sense, and vitamins, which contributed, at the most, negligible amounts of energy to diets, may profoundly affect the metabolism of energy producing nutrients.

Results are given of two series of experiments, each series consisting of parallel determinations on lots of five rats each, at body weights of approximately 100 and 240 gm., respectively. A different lot of five rats was used for each treatment at each age, and all data presented are averages representing five individuals.

Each series of experiments extended over several weeks' time, during spring and summer. With the advancement of the season there were naturally many changes in the climatic conditions to which the animals were subjected, and it is obvious that there were circumstances affecting the heat production which were not under control; but the results possess significance by comparison, apart from considerations as to their absolute validity.

Earlier experiments, the results of which are not presented, served to emphasize the fact that from the human point of view the life-span of the rat is indeed short, and that biological measurements on the same individual growing rats extending through a number of weeks may readily defeat their own purposes if it be assumed that the same background of physiological conditions continues throughout such an investigation.

Indeed it was found quite impracticable to maintain correct conditions for the comparison of diets by the use of the same individual animals as subjects through 6 to 10 weeks' observations. At the end of such a series of experiments the subjects were, in effect, not the same individuals as at the beginning.

A prominent difficulty encountered in this relation is that we do not have an entirely reliable means of computing the heat production of an animal to the basis of a standard live weight, as is necessary if the experimental treatment has produced changes in weight during the time required for the development of the desired response to the treatment.

Also, the previous feeding affects the heat production, and tends to confuse apparent dynamic effects in a series of observations with different diets and the same animals.

Further, rats do not eat as regularly as do some of the larger domestic animals and do not lend themselves as effectively to feeding at definite rates of intake in relation to the live weight; still the live weight does affect the heat production, and this fact must be dealt with in an effective manner.

At the expense of much loss of effort, therefore, the authors have learned that an advantageous way of providing for an extended series of dietary observations on growing rats is to select a different lot of animals of the same age and weight and having had the same preparatory treatment as the subjects for each unit of the program, and then to give them all the same quantities of feed.

Obviously, close consanguinity in the subjects and an adequate number of individuals in each experimental group are essential.

PROCEDURE

The general method of experimentation and the equipment used were approximately as described in a recent paper by Forbes, Kriss and Miller ('34), but with alterations in equipment as follows: a change of the water bath in which the respiration chamber was immersed to an air compartment with windows permitting observation of the animals; and the suspension of the respiration chamber from a work adder—thus providing effectively for the exclusion of data representing intervals of time during which there was any considerable activity of the experimental subjects.

Heat production was measured by the Haldane open-circuit respiratory quotient procedure in intervals of essentially complete rest during 7-hour periods in the respiration chamber, with the animal adjusted to and established on its dietary regime in the experiment.

Preliminary to the fasting heat measurement (determined to provide for the correction of the heat production of comparable animals to a basis of exactly the same live weight) the rats were given a maintenance ration for 5 days. Then an interval of 25 hours elapsed after the last feed was given before the actual fasting heat measurement was begun.

In preparation for the determination of the heat production from the basal and the supplemented diets the rats were thoroughly established on these planes of nutrition by preliminary feeding; then the same treatment was continued, during 1 day for the measurement of the heat production,

followed by 7 days for the collection of the excreta—for the determination of metabolizable energy.

The measurement of the heat production of a rat on the basal diet, or on the basal diet plus a supplement, was made after the animal had received at 8.00 A.M. one-half of its daily food allowance. Half an hour was allowed for eating and another half-hour for postprandial activity before the animal was placed in the respiration chamber at 9.00 A.M. The first hour's heat production was always discarded. The values for heat production actually used, therefore, represented the third to the eighth hours, inclusive, after feeding—minus periods of activity.

The experimental subjects were male, albino rats from the colony of this institute, in which a stock of a high degree of vigor and uniformity has been developed in the course of many generations of selective breeding.

The basal diets were composed of two-thirds ground wheat and one-third whole milk powder (Parlac) to which was added sodium chloride in the proportion of 0.5% of the wheat and milk powder.

The protein supplement was lean beef muscle, sterilized, dried at a low temperature, reduced to a powder and extracted with ether for 48 hours. The carbohydrate used was dextrin (Amidex) which was free from nitrogen, and the fat used was lard which was fed as purchased.

EXPERIMENTAL PROGRAM

The two experiments were conducted between March 10 and April 22, and between June 29 and August 9, 1938. The schedules of experimentation comprise tables 1 and 5, and the numerical data are presented in tables 2 to 4 and 6 to 8.

In addition to the metabolism experiments with the rats on feed, the 24-hour fasting heat production was determined as follows:

In experiment I this value was determined for the younger rats at an average age of 70 days, and average weight of

106 gm., on March 24th and 25th, this quota corrected to a standard weight of 100 gm. being 11.18 ± 0.09 kilo calories; and the fasting heat outgo was determined for the older rats at an average age of 113 days and an average weight of 237 gm., on May 6th to 12th, this value corrected to the basis of 240 gm. live weight being 19.40 ± 0.15 kilo calories.

TABLE 1
Schedule of experimentation. Experiment I

GROUP NUMBERS	DAILY FEEDING TREATMENT	AVERAGE AGE	AVERAGE WEIGHT	DATES OF RESPIRATION MEASUREMENTS
		days	*gm.*	
1	5 gm. basal diet	56	107	March 10–11
2	5 gm. basal diet + 3 gm. beef muscle protein	45	100	March 3–7
3	5 gm. basal diet + 3 gm. dextrin	46	100	Feb. 28–March 4
4	5 gm. basal diet + 1.24 gm. lard	49	101	March 3–7
5	5 gm. basal diet + 1.6 gm. mixture, b.m.p., d. and l.	45	100	March 2–5
6	9 gm. basal diet	99	238	April 22–28
7	9 gm. basal diet + 3 gm. beef muscle protein	87	240	April 12–25
8	9 gm. basal diet + 3 gm. dextrin	91	240	April 18–21
9	9 gm. basal diet + 1.24 gm. lard	92	240	April 16–26
10	9 gm. basal diet + 1.6 gm. mixture, b.m.p., d. and l.	92	242	April 16–22

TABLE 2
Utilization of nitrogen. Experiment I

GROUP NUMBERS	TREATMENTS	DAILY NITROGEN INTAKE	NITROGEN DIGESTED	NITROGEN OF URINE	NITROGEN RETENTION
		mg.	%	%	%
1	Basal diet	119	86.6	76.5	10.1
2	Basal diet + beef muscle protein	544	92.3	77.0	15.3
3	Basal diet + dextrin	124	85.5	27.4	58.1
4	Basal diet + lard	119	84.9	26.9	58.0
5	Basal diet + mixture, b.m.p., d. and l.	166	87.3	34.3	53.0
6	Basal diet	213	82.2	70.9	11.3
7	Basal diet + beef muscle protein	654	90.1	73.1	17.0
8	Basal diet + dextrin	211	78.2	47.4	30.8
9	Basal diet + lard	223	81.6	49.3	32.3
10	Basal diet + mixture, b.m.p., d. and l.	255	82.4	55.7	26.7

In experiment II the fasting heat production was determined for the younger rats at an average age of 67 days, and an average weight of 91 gm., on July 12th to 13th, this value corrected to the basis of 100 gm. live weight being 10.77 ± 0.22 kilo calories; and the same value was determined for the older rats at an average age of 93 days, and average weight of 229 gm. on August 4th to 11th, this value corrected to the basis of 240 gm. being 20.44 ± 0.18 kilo calories.

The quantities of the basal diets given were adequate for maintenance, and the supplements were fed superimposed upon these basal diets.

The quantities of the supplements given (3 gm. each of beef muscle protein and dextrin, 1.24 gm. of lard and 1.6 gm. of a mixture of the three) were such as contained approximately equal amounts of metabolizable energy; and the mixture of 62.5% lard and 18.75% each of dextrin and beef muscle protein supplied fat, carbohydrate and protein in the approximate proportions in which they are katabolized during fast.

DISCUSSION OF RESULTS

The data with reference to nitrogen utilization in tables 2 and 6 show that in both experiments and with both weights of rats, the diets supplemented by beef muscle protein, and therefore containing the maximum amounts of protein, were characterized by the maximum digestibility of their protein. These same diets led to the maximum percentage elimination of the dietary nitrogen in the urine; and among the supplements, the beef muscle protein led to the lowest percentage nitrogen utilization with both weights of rats in both experiments.

These observations are to be explained primarily by the excessive intake of protein by the rats which received the beef muscle preparation.

The digestibility of the protein of the basal diets and of the diets containing the non-nitrogenous supplements was naturally of the same general degree, and the digestibility of

TABLE 3

Metabolizable energy of diets and of supplements. Experiment I

GROUP NUMBERS	TREATMENTS	DAILY ENERGY INTAKE	ENERGY OF FECES	ENERGY OF URINE	METABOLIZABLE ENERGY	
					Total	Of supplement
		Cal.	%		%	%
1	Basal diet	21.89	9.7	4.0	86.3	
2	Basal diet + beef muscle protein	37.44	8.1	9.7	82.2	76.4
3	Basal diet + dextrin	33.31	6.5	2.7	90.8	99.2
4	Basal diet + lard	33.52	7.2	2.7	90.1	97.2
5	Basal diet + mixture, b.m.p., d. and l.	30.04	6.6	3.7	89.7	95.7
6	Basal diet	39.39	10.9	3.1	86.0	
7	Basal diet + beef muscle protein	54.50	9.3	8.1	82.6	73.9
8	Basal diet + dextrin	50.70	9.6	2.7	87.7	93.7
9	Basal diet + lard	51.40	10.0	3.3	86.7	89.0
10	Basal diet + mixture, b.m.p., d. and l.	51.61	9.4	3.7	86.9	90.1

TABLE 4

Daily heat production, dynamic effect and net energy. Experiment I

GROUP NUMBERS	TREATMENTS	DAILY HEAT PRODUCTION	DYNAMIC EFFECTS OF SUPPLEMENTS	NET ENERGY OF SUPPLEMENTS	
		Cal.	*% metabolizable*	*% metabolizable*	*Cal. per gram*
1	Basal diet	13.05 ± 0.04[1]			
2	Basal diet + beef muscle protein	21.37 ± 0.14[1]	70.1	29.9	1.19
3	Basal diet + dextrin	19.22 ± 0.21[1]	54.5	45.5	1.72
4	Basal diet + lard	16.19 ± 0.15[1]	27.8	72.2	6.59
5	Basal diet + mixture, b.m.p., d. and l.	17.63 ± 0.16[1]	39.4	60.6	4.41
6	Basal diet	23.34 ± 0.21[2]			
7	Basal diet + beef muscle protein	30.37 ± 0.15[2]	62.9	37.1	1.38
8	Basal diet + dextrin	27.97 ± 0.12[2]	43.7	56.3	1.99
9	Basal diet + lard	26.50 ± 0.17[2]	29.6	70.4	6.06
10	Basal diet + mixture, b.m.p., d. and l.	26.62 ± 0.19[2]	29.8	70.2	4.83

[1] Corrected to standard weight of 100 gm.

[2] Corrected to standard weight of 240 gm.

the protein of the diets containing the mixture of the supplements (one of them, beef protein) was slightly higher than that of the diets containing no beef protein.

The percentage retention of the dietary nitrogen was high in the periods in which the nitrogen intake was relatively low and the intake of non-nitrogenous nutriment relatively high, that is, in periods 3, 4, 5, 8, 9 and 10, in which the dextrin or the lard, or the two together, tended to spare the protein of the basal diet from katabolism for energy production.

TABLE 5

Schedule of experimentation. Experiment II

GROUP NUMBERS	DAILY FEEDING TREATMENT	AVERAGE AGE	AVERAGE WEIGHT	DATES OF RESPIRATION MEASUREMENTS
		days	*gm.*	
1	5 gm. basal diet	54	96	June 29–30
2	5 gm. basal diet + 3 gm. beef muscle protein	44	100	June 20–28
3	5 gm. basal diet + 3 gm. dextrine	48	101	June 22–25
4	5 gm. basal diet + 1.24 gm. lard	48	101	June 21–27
5	5 gm. basal diet + 1.6 gm. mixture, b.m.p., d. and l.	50	100	June 22–28
6	9 gm. basal diet	79	235	July 21–28
7	9 gm. basal diet + 3 gm. beef muscle protein	82	241	July 25–Aug. 3
8	9 gm. basal diet + 3 gm. dextrin	81	240	July 22–Aug. 3
9	9 gm. basal diet + 1.24 gm. lard	85	241	July 26–Aug. 9
10	9 gm. basal diet + 1.6 gm. mixture, b.m.p., d. and l.	83	242	July 25–Aug. 9

The data representing the metabolizable energy of the diets and of the supplements comprise tables 3 and 7.

The values for urinary energy, as given, are as corrected for nitrogen retention, that is, as though the subjects had been in nitrogen equilibrium. This is for the purpose of recognizing that a portion of the body increase of protein is non-metabolizable in the sense that it cannot be transformed into heat but, in the event that the stored substance is katabolized, is eliminated in the urine in compounds containing potential energy.

The notable points in relation to the metabolizable energy of the diets are 1) the relative constancy of the percentage of the food energy appearing in the feces, 2) the relatively high percentage of the food energy appearing in the urine in those periods in which the basal ration was supplemented with the beef muscle protein, 3) the relatively low metabolizability of the food energy in the same periods (2 and 7), and 4) among the supplements, the relatively low metabolizability of the energy of the beef muscle protein preparation.

TABLE 6

Utilization of nitrogen. Experiment II

GROUP NUMBERS	TREATMENTS	DAILY NITROGEN INTAKE	NITROGEN DIGESTED	NITROGEN OF URINE	NITROGEN RETENTION
		mg.	%	%	%
1	Basal diet	128	85.2	76.6	8.6
2	Basal diet + beef muscle protein	532	92.1	77.3	14.8
3	Basal diet + dextrin	125	85.6	38.4	47.2
4	Basal diet + lard	128	85.9	35.9	50.0
5	Basal diet + mixture, b.m.p., d. and l.	167	87.4	49.1	38.3
6	Basal diet	230	83.0	62.2	20.8
7	Basal diet + beef muscle protein	632	89.1	67.7	21.4
8	Basal diet + dextrin	226	81.0	39.8	41.2
9	Basal diet + lard	226	80.1	43.8	36.3
10	Basal diet + mixture, b.m.p., d. and l.	273	83.2	52.8	30.4

The main results of the experiments, the heat production from the diets, and the dynamic effects and net energy values of the supplements, comprise tables 4 and 8.

In seven instances among the total of eight, the dynamic effects of the supplements were higher for the 100-gm. rats than for the 240-gm. rats.

In both experiments and with both weights of rats, the dynamic effects of the individual supplements were in the same order—protein highest, carbohydrate intermediate and fat lowest.

TABLE 7

Metabolizable energy of diets and supplements. Experiment II

GROUP NUMBERS	TREATMENTS	DAILY ENERGY INTAKE	ENERGY OF FECES	ENERGY OF URINE	METABOLIZABLE ENERGY	
					Total	Of supplement
		Cal.	%	%	%	%
1	Basal diet	22.07	8.8	3.5	87.7	
2	Basal diet + beef muscle protein	36.47	7.9	9.8	82.3	74.0
3	Basal diet + dextrin	33.41	6.6	2.6	90.8	96.9
4	Basal diet + lard	33.53	6.7	2.5	90.8	96.9
5	Basal diet + mixture, b.m.p., d. and l.	33.79	6.9	3.4	89.7	93.4
6	Basal diet	39.73	10.7	4.4	84.9	
7	Basal diet + beef muscle protein	54.34	9.3	8.0	82.7	76.4
8	Basal diet + dextrin	50.74	9.3	3.4	87.3	95.6
9	Basal diet + lard	51.01	9.8	3.4	86.8	93.4
10	Basal diet + mixture, b.m.p., d. and l.	51.57	9.2	3.8	87.0	93.8

TABLE 8

Daily heat production, dynamic effect and net energy. Experiment II

GROUP NUMBERS	TREATMENTS	DAILY HEAT PRODUCTION	DYNAMIC EFFECT OF SUPPLEMENTS	NET ENERGY OF SUPLEMENTS	
		Cal.	*% metabolizable*	*% metabolizable*	*Cal. per gram*
1	Basal diet	13.27 ± 0.14[1]			
2	Basal diet + beef muscle protein	20.21 ± 0.23[1]	65.0	35.0	1.24
3	Basal diet + dextrin	17.81 ± 0.18[1]	41.3	58.7	2.15
4	Basal diet + lard	16.59 ± 0.14[1]	29.9	70.1	6.28
5	Basal diet + mixture, b.m.p., d. and l.	16.40 ± 0.06[1]	28.6	71.4	4.89
6	Basal diet	25.53 ± 0.40[2]			
7	Basal diet + beef muscle protein	31.16 ± 0.17[2]	50.4	49.6	1.85
8	Basal diet + dextrin	29.32 ± 0.13[2]	36.0	64.0	2.25
9	Basal diet + lard	28.49 ± 0.48[2]	28.0	72.0	6.12
10	Basal diet + mixture, b.m.p., d. and l.	28.53 ± 0.18[2]	26.9	73.1	5.07

[1] Corrected to standard weight of 100 gm.

[2] Corrected to standard weight of 240 gm.

In the four comparisons provided by the two experiments, and the two weights of rats, the dynamic effect of the mixed supplement was in each case much lower than could have been computed from the individual values as determined for the protein, carbohydrate and fat of which the supplement was composed; and in three comparisons among the four the dynamic effect of the mixed supplement was either as low as or lower than the dynamic effect of the lard, which was in all cases lower than that of the carbohydrate or of the protein.

There is, therefore, a nutritive supplementation of the three main classes of nutrient compounds in combination which profoundly influences their dynamic effects.

In other words, the dynamic effects of individual nutrients are not significant with respect to combined nutrients, as in all nutritive practice, though popular impression is to the contrary. If such values are not to mislead they must be used in a critically guarded manner, for they have no general nutritive significance.

The net energy of the beef muscle protein is the lowest because of its low metabolizability and high heat increment; dextrin has a higher net energy value than has beef muscle protein, while the value for lard is much higher still.

As the evidence stands—the net energy values for the beef muscle protein and the dextrin were higher, and the value for the lard was lower, for the older than for the younger rats.

SUMMARY

The dynamic effects and net energy values of beef muscle protein, dextrin and lard were determined, separately and in combination, by feeding superimposed upon an approximately complete basal diet.

The subjects were growing, male, albino rats, and heat was measured by the open-circuit Haldane procedure.

The point of view of the experimenters is that even though a nutrient is fed alone it is never utilized alone, and that the

combination in which it is metabolized largely determines its dynamic and net energy values.

Among the individual supplements the dynamic effect of the beef muscle protein was the highest, that of dextrin was intermediate, and that of lard the lowest; while the dynamic effect of a mixture of the three nutrients was in all cases much lower than would be a value computed from the individual measurements for the three components. Dynamic effects as determined for individual nutrients, therefore, are not significant with reference to combined nutrients.

In seven instances among eight the dynamic effects were higher for the 100-gm. than for the 240-gm. rats.

The net energy of the beef muscle protein was the lowest because of its low metabolizability and high heat increment; dextrin has a higher net energy value than has beef muscle protein, while the value for lard is much higher still.

LITERATURE CITED

FORBES, E. B., W. W. BRAMAN, MAX KRISS ET AL. 1930 Further studies of the energy metabolism of cattle in relation to the plane of nutrition. J. Agr. Research, vol. 40, no. 1, 37–78, see pp. 53, 58 and 77.

FORBES, E. B. 1933 The law of maximum normal nutritive value. Science, vol. 77, no. 1995, pp. 306–307.

FORBES, E. B., MAX KRISS AND R. C. MILLER 1934 The energy metabolism of the albino rat in relation to the plane of nutrition. J. Nutrition, vol. 8, pp. 535–552.

KRISS, MAX, E. B. FORBES AND R. C. MILLER 1934 The specific dynamic effects of protein, fat and carbohydrate as determined with the albino rat at different planes of nutrition. J. Nutrition, vol. 3, pp. 509–534.

RUBNER, M. 1902 Die Gesetze des Energieverbrauches bei der Ernährung. Leipzig u. Wien.

THE VITAMIN D REQUIREMENT OF YOUNG TURKEYS

T. H. JUKES AND T. D. SANFORD

Division of Poultry Husbandry, University of California, Davis, and the Biological Laboratories of the F. E. Booth Co., Berkeley, California

TWO FIGURES

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INTRODUCTION

It was observed by Scott, Hughes and Loy ('32) that the vitamin D requirement of young turkeys was higher than that of chicks. Baird and Greene ('35) reported that chicks required 18 U.S.P. units of vitamin D per 100 gm. of diet and turkeys required 60 to 70 units. The experimental period lasted 12 weeks, and the data indicated that the requirement of turkeys for vitamin D expressed in units per 100 gm. of diet, was greater for the first 4 weeks of their lives than for the ensuing 8 weeks. Baird and Greene found that turkeys receiving 0.35% of fortified cod liver oil in the diet had an average tibial ash content of 43.9% at 4 weeks. Higher levels of the oil, which contained 250 units (U.S.P. X, revised 1934) of vitamin D per gram, were not fed.

Recently the A.O.A.C. chick unit (Association of Official Agricultural Chemists, 'Methods of Analysis,' 1935, p. 351) has come into wide use in testing sources of vitamin D used in poultry feeding. The present investigation was initiated with the intention of finding the vitamin D requirement of young turkeys in terms of A.O.A.C. chick units.

EXPERIMENTAL

A. Experiments with turkeys

In a preliminary experiment, a group of turkeys was kept for 4 weeks on a diet to which no vitamin D was added. At the end of the experimental period the average tibial ash content was found to be 29.2%. A second group received a supplement of 0.3 gm. of a fish oil blend stated to supply 400 A.O.A.C. chick units of vitamin D per gram, to 100 gm. of diet. The tibial ash content of this group was 48.0%. It seemed from these results that Baird and Greene ('35) might not have reached the level of vitamin D necessary for maximal calcification.

In the next experiment, the following basal diet was used for turkeys (diet PD): ground yellow corn, 25%; ground barley, 25; ground wheat 7; wheat bran, 15; sardine meal, fat extracted, 17.5; dried skim milk, 5; dehydrated alfalfa meal, 7.5; ground limestone, 1.5; steamed bonemeal, 1; and salt, 0.5. It contained approximately 23% of protein, 2.0% of calcium, and 1.0% of phosphorus. Except for the omission of the vitamin D supplement, the diet was a typical turkey starting diet.

Turkeys were hatched from eggs laid by the flock at the Poultry Division, Davis. The parent birds were kept in outside yards and were not housed; their diet consisted of approximately equal parts of whole grains and a mash containing 0.5% of sardine oil. The sardine oil was stated to contain a minimum of 100 A.O.A.C. chick units of vitamin D per gram. Undoubtedly the parent birds received a large additional supply of vitamin D from direct sunlight. Turkey poults were removed from the incubator on the day after hatching and fed the experimental diets immediately. The birds were kept in electrically heated, battery brooders with wire floors in a room from which daylight was excluded. From eleven to twenty birds were used in a group. Several varieties and crosses were used with the Bronze variety predominating. The birds were weighed at hatching and weekly thereafter.

They were killed on the twenty-eighth day, and the tibiae were removed, cleaned and placed in 95% ethanol. The bones were then extracted in a Soxhlet apparatus for 24 hours with anhydrous isopropanol; then they were turned end for end in the extractors and the extraction continued for a further 24 hours. Following this, the bones were extracted with isopropyl ether for 30 hours and were similarly reversed halfway through the ether extraction. The extracted bones were placed individually in tared Sillimanite crucibles, dried at 110° for 24 hours and weighed. Drying was repeated for 36 hours, following which the crucibles were reweighed. The crucibles were then placed in a cold electric muffle furnace, the temperature of which was raised to 750° during a period of 1 hour. This temperature was maintained for 2 hours, and the weights of the residues from the combustion were used to calculate the percentage of ash.

Sources of vitamin D were diluted with cottonseed oil [1] and mixed with the diets so that oil was added to all diets at the rate of 2 gm. to 98 gm. of diet. Reference cod liver oil, control 1-38-D containing 95 U.S.P. XI vitamin D units in 1 gm. of the oil, was purchased from the Board of Trustees of the United States Pharmacopeial Convention. The A.O.A.C. chick unit, by definition, is the potency of one unit of U.S.P. reference cod liver oil measured with chicks. Hence the use of this oil should enable a direct determination of the vitamin D requirement of turkeys in terms of A.O.A.C. chick units. The other oil used in the investigation was a fish oil blend [2] consisting of sardine oil fortified in vitamins A and D from the untreated liver oils of various fish to a potency of approximately 400 A.O.A.C. chick units of vitamin D and 3000 U.S.P. units of vitamin A per gram. It will be referred to as 'fish oil 1.'

In the first experiment eight groups of poults were used. Group 1 received basal diet PD without supplementation with

[1] 'Wesson oil.'
[2] 'Sardilene 400.'

vitamin D. The other groups received supplements as shown in table 1.

It was expected that the diet received by group 6 would provide sufficient vitamin D for maximal calcification, and when the experiment was planned, groups 7 and 8 were included merely as 'positive control' groups. However, the results made it evident that 100 units of vitamin D added in the form of reference cod liver oil per 100 gm. of diet were far from sufficient for maximal growth and calcification. Three-tenths per cent of fish oil 1 was also insufficient, since growth and calcification were significantly greater when 0.5% was used. In figure 1 the calculated straight line of best fit has been plotted for the bone ash of groups 1 to 6 by use of the method of least squares. When this line is extrapolated, a value of 46.8% bone ash, corresponding to group 7, gives a vitamin D value of 189 'units' per 100 gm. of diet, corresponding to 630 'units' per gram of fish oil 1. The 'unit' obtained by this extrapolation is based on a comparison of U.S.P. reference cod liver oil with a source of vitamin D of unknown potency using the turkey as a test animal. Such a 'unit' may be referred to for convenience as a 'turkey unit,' and is an expression of the response obtained in turkeys from 1 U.S.P. unit of reference cod liver oil. The straight line extrapolation is not necessarily justifiable, since Massengale and Bills ('36) demonstrated that the response curve for chicks had a progressively diminishing slope and varied in shape with the source of vitamin D. If the response curve for turkeys diminished in slope in the region beyond the points corresponding to groups 1 to 6, an even higher value would be obtained for the vitamin D content of diet 7.

Curve A, figure 1, indicates that unit increase in calcification in chicks in the experiments of Massengale and Bills ('36) was produced by one-tenth of the increase in vitamin D unitage required to produce unit increase of calcification in turkeys in the present investigation. This statement applies only to a range of values in the lower and linear region of the response curve.

TABLE 1

Summarized results of first experiment with turkeys, hatched May 10, 1938 showing the relation of body weight, tibial weight and tibial ash to level of vitamin D in the diet. The birds were sacrificed at an age of 4 weeks

Group	Supplement to 100 gm. of basal diet PD			Number of birds at 4 weeks		Average body weight at 4 weeks			Average weight of dry fat-free left tibiae			Average ash of tibiae		
				Males	Females	Males	Females	Average of both sexes	Males	Females	Average of both sexes	Males	Females	Average of both sexes
	gm.	Oil	Units of vitamin D			gm.	gm.	gm.	gm.	gm.	gm.	%	%	%
1	None		0	7	9	186	173	180	0.592	0.508	0.550	28.27	31.22	29.74
2	0.21	CLO [1]	20	6	8	210	198	204	0.600	0.583	0.592	32.25	31.76	32.00
3	0.42	CLO	40	4	11	242	244	243	0.676	0.673	0.674	31.62	34.96	33.29
4	0.63	CLO	60	3	11	232	271	252	0.665	0.771	0.718	32.63	36.28	34.46
5	0.84	CLO	80	5	10	239	220	230	0.748	0.675	0.712	34.98	38.31	36.64
6	1.05	CLO	100	8	6	304	264	284	0.883	0.789	0.836	37.19	40.92	39.06
7	0.30	Fish oil 1		6	11	302	308	305	0.925	1.033	0.979	45.54	48.07	46.80
8	0.50	Fish oil 1		9	6	359	336	348	1.250	1.144	1.197	48.90	49.17	49.03

[1] CLO = U.S.P. reference cod liver oil.

In the second experiment, levels of 0.05, 0.1 and 0.2% of fish oil 1 were selected to be within the range covered by the levels of U.S.P. cod liver oil used, which were 0, 0.63 and 1.26% supplying respectively 0, 60 and 120 U.S.P. units of vitamin D per 100 gm. of diet. By this means, it was hoped to obtain a close evaluation of the potency of fish oil 1 as compared with reference cod liver oil using the turkey as a test animal.

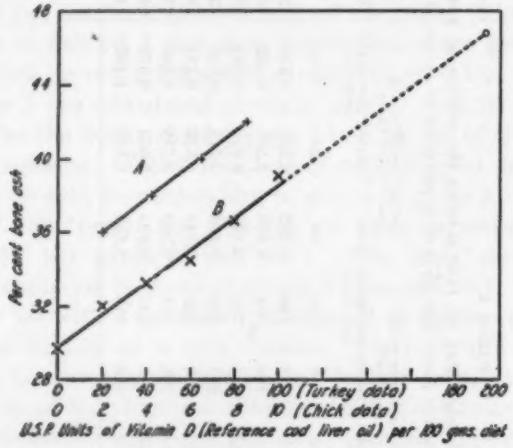

Fig. 1 Curve A, shown for comparison, was plotted from a short range of the data of Massengale and Bills ('36) for the femoral ash of chicks at 45 days of age, using a horizontal scale of one-tenth the value of the scale used for curve B. Curve B was plotted from the data of experiment 1 for the tibial ash of turkeys at 4 weeks of age. The points marked 'x' are the values obtained with U.S.P. reference cod liver oil. The calculated straight line of best fit was plotted for these points and was extrapolated as a dotted line to the point shown by a circle, which corresponds to the bone ash value of group 7, receiving 0.3 gm. of fish oil 1 per 100 gm. of diet.

Levels of 0.3 and 0.4% of fish oil 1 were included in the series in order to obtain further information on the level of this oil required for maximal growth and calcification. The results are shown in table 2. Growth was slower in groups 15 and 16 as compared with groups 7 and 8, probably because the poults in the second experiment were hatched later in the

TABLE 2

Summarized results of second experiment with turkeys, hatched June 21, 1938

Group	Supplement to 100 gm. of basal diet PD			Number of birds at 4 weeks		Average body weight at 4 weeks			Average weight of dry fat-free left tibiae			Average ash of tibiae		
				Males	Females	Males	Females	Average of both sexes	Males	Females	Average of both sexes	Males	Females	Average of both sexes
	gm.	Oil	Units of vitamin D			gm.	gm.	gm.	gm.	gm.	gm.	%	%	%
9	None		0	4	11	194	181	187	0.544	0.520	0.532	30.05	30.19	30.12
10	0.05	Fish oil 1		4	10	218	200	209	0.555	0.519	0.537	34.05	34.66	34.36
11	0.10	Fish oil 1		7	5	352	214	233	0.695	0.599	0.647	36.74	36.76	36.75
12	0.63	CLO [1]	60	5	7	281	225	253	0.811	0.619	0.715	37.92	38.04	37.98
13	0.20	Fish oil 1		8	5	267	235	251	0.819	0.727	0.773	42.31	44.86	43.58
14	1.26	CLO [1]	120	3	7	258	265	262	0.779	0.830	0.804	42.47	44.26	43.36
15	0.30	Fish oil 1		3	8	261	281	271	0.765	0.818	0.792	46.03	46.29	46.16
16	0.40	Fish oil 1		7	4	300	256	278	1.004	0.841	0.922	50.00	50.02	50.01

[1] CLO = U.S.P. reference cod liver oil.

season and hence grew more slowly (Asmundson and Lloyd, '36).

Acute symptoms of vitamin D deficiency were observed in turkeys on the basal diet. At 14 days of age the birds manifested a tendency to squat. After the twenty-first day, some of the birds were unable to stand. Four birds in group 1 died during the fourth week. No birds in any of the other groups, including group 9, died during the fourth week. Three birds in group 1 had bone ash values of less than 26%.

In order to evaluate the potency of fish oil 1, a calculated straight line of best fit was plotted for the bone ash values obtained from groups 9, 12 and 14 using the same coordinates as in figure 1. The bone ash percentages of groups 10, 11 and 13 when plotted on this line gave respective values of 34, 57 and 118 'turkey units' of vitamin D per 100 gm. of diet. These values correspond to 680, 570 and 590; mean 613 $\pm$ 48 'turkey units' of vitamin D per gram of fish oil 1. The first experiment had indicated roughly that fish oil 1 contained about 630 'turkey units' of vitamin D per gram. The values obtained in both experiments with turkeys indicated that fish oil 1 had an unexpectedly high vitamin D content, in view of the fact that the oil supposedly contained about 400 chick units per gram. It hence was thought desirable to test fish oil 1 with chicks in order to find the number of chick units of vitamin D per gram.

B. Evaluation of fish oil 1 with chicks

Single-comb White Leghorn chicks were used. In experiment 3 the chicks were hatched from eggs obtained from the University flock at Berkeley. The parent birds received a diet consisting of approximately equal parts of whole grains and a mash containing 0.3% of a fish oil stated to contain a minimum of 400 A.O.A.C. chick units of vitamin D per gram. The parent birds also had access to yards giving them contact with direct sunlight. The other experimental details were as described for turkeys. Diet PD was used as the basal chick diet. The results are summarized in table 3.

The comparatively high level of bone ash in group 17 receiving basal diet PD indicates that the diet did not produce acute rickets in chicks in contrast to the results with turkeys. The values obtained for body weight and tibial ash do not show the consistent trends obtained with turkeys. For evaluation of fish oil 1, the same procedure was used as in experiment 2, a straight line of best fit being plotted from the bone ash values obtained from groups 17, 18, 19 and 20. The bone ash percentages of groups 22, 23 and 24 when plotted on this line gave respective values of 6.1, 13.1 and 12.7 chick units of

TABLE 3

Comparison of vitamin D potency of U.S.P. reference cod liver oil with fish oil 1, using chicks as test animals and diet PD as basal diet

Group	Supplement to 100 gm. of basal diet			Number of birds at 4 weeks		Average body weight at 4 weeks	Average ash of tibiae
				Males	Females		
	gm.	Oil	Units of vitamin D			gm.	%
17	None		0	5	7	168	39.9
18	0.105	CLO[1]	10	7	5	178	42.8
19	0.157	CLO	15	4	7	165	44.9
20	0.210	CLO	20	8	4	184	44.5
21	0.262	CLO	25	5	6	181	46.1
22	0.0125	Fish oil 1		6	4	195	41.8
23	0.0250	Fish oil 1		6	6	167	43.5
24	0.0375	Fish oil 1		3	9	169	43.4
25	0.0500	Fish oil 1		6	6	178	46.3
26	0.1000	Fish oil 1		6	5	186	48.1

[1] CLO = U.S.P. reference cod liver oil.

vitamin D per 100 gm. of diet. These values correspond to 488, 524 and 339; mean 450 $\pm$ 80 units of vitamin D per gram of fish oil 1. Groups 21, 25 and 26 were omitted from the calculation because the bone ash values were in the upper and non-linear region of the response curve (Massengale and Bills, '36). The sex ratios of the groups in experiment 3 fell within the limits shown by Massengale and Bills ('36) to be inconsequential with respect to sex error of bone ash.

The results indicated that fish oil 1 contained fewer 'chick units' than 'turkey units' of vitamin D per gram when

measured by means of the same basal diet and reference cod liver oil. However, the results with chicks in experiment 3 showed a wide variation, and it was hence thought advisable to assay fish oil 1 by means of a modified A.O.A.C. method in the expectation of obtaining a more accurate evaluation of the potency of fish oil 1 in terms of 'chick units.' The following basal diet was used: ground yellow corn, 56%; wheat middlings, 25; casein, 10; dried skim milk, 5; dried yeast, 1; bone meal, 1; calcium carbonate, 1; sodium chloride, 1. Chicks from a commercial hatchery received the basal diet for 10

TABLE 4

Comparison of vitamin D potency of U.S.P. reference cod liver oil with fish oil 1 using chicks as test animals with a modified A.O.A.C. basal diet

Group	Supplement to 100 gm. of basal diet			Number of birds at 31 days	Average body weight at 31 days	Average ash of tibiae
	gm.	Oil	Units of vitamin D		gm.	%
27	None		0	12	195	33.9
28	0.052	CLO[1]	5	13	193	35.5
29	0.105	CLO	10	14	225	38.5
30	0.157	CLO	15	14	247	40.0
31	0.210	CLO	20	15	234	44.4
32	0.262	CLO	25	14	245	44.0
33	0.020	Fish oil 1		15	226	37.9
34	0.030	Fish oil 1		14	225	39.0
35	0.040	Fish oil 1		15	223	42.4

[1] CLO = U.S.P. reference cod liver oil.

days and then were fed the test diets for the next 21 days, after which they were killed and the bone ash content of the tibiae determined as described above. The results are summarized in table 4. For evaluation of fish oil 1, the same procedure was used as in experiment 3 except that the straight line of best fit was plotted from the bone ash values obtained from groups 27, 28, 29 and 30. The bone ash percentages of groups 33, 34 and 35 when plotted on this line gave respective values of 9.7, 12.2 and 20.2 chick units of vitamin D per 100 gm. of diet, corresponding to 485, 407 and 505; mean 466 ± 42 units of vitamin D per gram of fish oil 1. Hence

experiment 4 gave a value for fish oil 1 similar to that obtained in experiment 3.

DISCUSSION

It has been demonstrated by Bills, Massengale, Imboden and Hall ('37) and Bills, Massengale, Hickman and Gray ('38) that fish oils may contain varying mixtures of the several forms of vitamin D. A comparison of the efficiency of these various forms when made with rats is different from a comparison made with chicks. This fact led Bills, Massengale, Imboden and Hall ('37) to speak of the 'efficacy ratio' of a fish oil as a measure of the comparative response of rats and chicks to 1 unit of the given fish oil, the basis of standardization being in each case a reference cod liver oil which was assigned a value of 100. The efficacy ratio (perhaps a better term would be 'efficacy quotient') of a fish oil is an expression of the resultant of the efficacy ratios of the various forms of vitamin D contained in the oil. The fact that the efficacy ratio of a fish oil often departs far from the value of 100 has led to the widespread adoption of the chick as a test animal for sources of vitamin D which are to be used in feeding chickens. The present investigation indicates the possibility of the existence of a difference between chicks and turkeys in their response to various sources of vitamin D, and suggests that inaccuracies may be caused by use of the chick test in evaluating sources of vitamin D to be used in feeding turkeys. Fortunately the issue is not of great practical importance, since a margin of safety may be easily provided in rations fed to young turkeys, and older birds commonly receive most or all of their vitamin D requirement from direct sunlight.

The possibility remains that the difference in response observed between chicks and turkeys was due to differences in the amount of uptake from the gut. No evidence is available on this point, although Klein and Russell ('31) have shown that the low efficacy of irradiated ergosterol for chicks is not due to a poor uptake.

The efficacy ratio of a fish oil, determined by the rat: chick method, varies with the degree of calcification produced (Bills and co-workers, '37). This may also be the case when chicks and turkeys are compared, but the data in the present investigation are not sufficiently extensive to illustrate the point.

A straight line appeared to be the best form of curve to fit to the bone ash values obtained from groups 1 to 6 (fig. 1). This was not unexpected, since Massengale and Bills ('36) using chicks observed that 'beginning at a threshold corresponding to the first definite betterment of the ricketic bone,

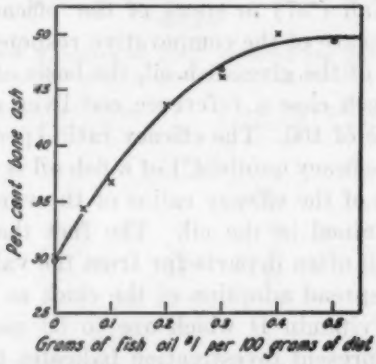

Fig. 2 Response curve obtained by plotting tibial ash values of turkeys at 4 weeks of age against levels of fish oil 1. Circles represent values obtained in experiment 1, crosses represent values obtained in experiment 2. One gram of fish oil 1 supplied about 620 'turkey units' of vitamin D when compared with U.S.P. reference cod liver oil, using the turkey as a test animal. The curve is the parabola of best fit, calculated by the method of least squares, and produced in a straight line beyond its point of inflexion (49.6% bone ash). The results indicate that maximal calcification necessitated levels of vitamin D in the neighborhood of 250 'turkey units' per 100 gm. of diet.

and continuing halfway toward normal bone, the relation of units to ash is essentially linear." A straight line was also used for the evaluation of fish oil 1 in the other experiments because the points used for evaluation fell in the linear region. When the effect of higher levels of vitamin D on turkeys was examined by means of fish oil 1, a parabola (fig. 2) seemed to correspond well to the observed data.

The turkey was found to be a sensitive test animal for the study of vitamin D. Bone ash values extended over a range of from 30 to 50% when a range of vitamin D levels was used which extended from 0 to over 200 units per 100 gm. of diet. Moreover, the diet was not distorted for the purpose of rachitogenesis; indeed, the bone ash value of 39.9% obtained from group 17 of chicks (table 4) on the turkey basal diet indicated that the diet failed to produce acute rickets in chicks, which further emphasizes the high vitamin D requirement of young turkeys. The favorable levels of calcium and phosphorus in the turkey basal diet may have accounted for the mild nature of the rickets in group 17 (compare Dols, '36).

An examination of the data in tables 1 and 2 shows that the average bone ash of the females was greater than that of the males in fifteen of the sixteen groups, the mean difference being 1.56 ± 0.38. This difference cannot be explained on the basis of slower growth leading to greater calcification because the average body weight of the females was actually greater than that of the males in five of the sixteen groups, and the average weight of the tibiae of the females was greater than that of the males in four groups. This sex difference in rate of calcification may have some relation to the observation of Marsden ('31) that male turkeys tended to develop deformed breast bones more readily than females.

The relation of growth of turkeys to the level of vitamin D fed was quite marked. In the first experiment the birds which received the highest level of vitamin D supplementation weighed 93% more than the birds on the basal diet at 4 weeks. Intervening levels of vitamin D produced growth responses which indicated some correlation between growth and calcification.

Massengale and Bills ('36) regard a bone ash of between 46 and 47% in chickens as normal under the conditions of their experiments. In the present communication, no attempt is made to define the 'normal' level of bone ash for turkeys, but it may be noted that in both of the experiments involving turkeys, the highest body weight at 4 weeks accompanied the

highest level of calcification, which was 49.03% in the first experiment and 50.01% in the second.

SUMMARY

1. An investigation of the vitamin D requirement of young turkeys was made by measurement of the ash content of the tibiae at 4 weeks of age as influenced by various levels of vitamin D in the diet.

2. Under the conditions of the experiment, the highest bone ash was in the neighborhood of 50%.

3. U.S.P. reference cod liver oil produced unsatisfactory growth and calcification when added at a level of 120 units (U.S.P. or A.O.A.C.) per 100 gm. of diet. Higher levels were not fed.

4. A linear relationship between vitamin D level and bone ash was observed in an experiment in which reference cod liver oil was added at levels ranging from 20 to 100 units per 100 gm. of diet.

5. Growth rate of turkeys was markedly influenced by vitamin D; in one experiment the birds receiving an ample supply of the vitamin weighed 93% more than the birds on the basal diet at 4 weeks.

6. Satisfactory growth and calcification in complete absence of sunlight were produced when turkeys received 0.4 gm. of a fish oil blend per 100 gm. of diet. Significantly poorer calcification was produced when 0.3 gm. was used. The oil was tested with chicks and was found to supply approximately 450 units of vitamin D per gram when the turkey basal diet was fed, and 466 units when a modified A.O.A.C. basal diet was used, based on a value of 95 units of vitamin D per gram for U.S.P. reference cod liver oil.

7. The fish oil blend functioned as a source of vitamin D that was more effective, chick unit for chick unit, than reference cod liver oil for turkey poults. The average turkey: chick efficacy ratio of the fish oil blend was about 140 (cod liver oil = 100). Hence the vitamin D requirement of turkeys could not be precisely stated in terms of A.O.A.C. chick units,

since the possibility of a differential species response between chicks and turkeys is not considered by such an expression. A value of 200 A.O.A.C. chick units of vitamin D per 100 gm. of diet may be taken as an approximation of the requirement of turkeys for the first 4 weeks to produce maximal calcification in the complete absence of sunlight.

LITERATURE CITED

ASMUNDSON, V. S., AND W. E. LLOYD 1936 Effect of season on the growth and feed consumption of turkeys. Poultry Sci., vol. 15, p. 186.

BAIRD, F. D., AND D. J. GREENE 1935 The comparative vitamin D requirements of growing chicks, turkeys and pheasants. Poultry Sci., vol. 14, p. 70.

BILLS, C. E., O. N. MASSENGALE, K. C. D. HICKMAN AND E. LEB. GRAY 1938 A new vitamin D in cod liver oil. J. Biol. Chem., vol. 126, p. 241.

BILLS, C. E., O. N. MASSENGALE, M. IMBODEN AND H. HALL 1937 The multiple nature of the vitamin D of fish oils. J. Nutrition, vol. 13, p. 435.

DOLS, M. J. L. 1936 Influence of the calcium and phosphorus ratios and the calcium and phosphorus contents in the basal food on the need for vitamin D for chicks. Arch. néerland. physiol., vol. 21, p. 554.

KLEIN, D., AND W. C. RUSSELL 1931 The fate of the antirachitic factor in the chicken. I. The antirachitic factor balance in the growing chick. J. Biol. Chem., vol. 93, p. 693.

MARSDEN, S. J. 1931 Progress report of turkey investigations at the United States range livestock experiment station. U. S. Dept. of Agr. leaflet, 28 pp.

MASSENGALE, O. N., AND C. E. BILLS 1936 A quantitative method for the assay of vitamin D with chickens. J. Nutrition, vol. 12, p. 429.

SCOTT, H. M., J. S. HUGHES AND H. W. LOY 1932 Rickets in young turkeys. Poultry Sci., vol. 11, p. 177.

THE EFFECT OF APPLE CONSTITUENTS ON THE RETENTION BY GROWING RATS OF LEAD CONTAINED IN SPRAY RESIDUES[1]

J. B. SHIELDS, H. H. MITCHELL AND W. A. RUTH
Division of Animal Nutrition, and Department of Horticulture, University of Illinois, Urbana

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The value of any food material depends both upon the nutrients contained in it and their availability in covering animal requirements, and upon the physiological effect of the food. Under the latter heading is included the effects of the food material in modifying body processes: digestion, heat production, resistance to disease, etc. Most foods are evaluated entirely upon their content of available nutrients, but a few, although they may rate high according to nutrient content, are also of value in the diet because of some specific physiological effect on the body. Among such foods are apple, banana, wheat bran, lactose, gelatin and liver.

Although in many respects the apple is a good food on the basis of its nutrient content (Todhunter, '37), it is not preeminent with respect to any one nutrient. However, the apple has been found to possess many remarkably favorable properties quite aside from its value in covering the nutritive requirements of the body. Its efficacy in the prevention and treatment of diarrheal conditions and other intestinal disorders, particularly in infants, has been frequently demonstrated (Manville, Bradway and McMinis, '37; Manville, '38).

[1] This investigation was conducted under the supervision of a Committee on the Physiological Effects of Spray Chemicals, appointed by the director of the Agricultural Experiment Station and consisting of the following members: H. H. Mitchell, W. A. Ruth, W. P. Flint and Julia P. Outhouse.

This favorable action has been ascribed with much reason to the pectins contained in apple (Baumann and Forschner-Böke, '34; Malyoth, '34); the finding that a commercial pectin is much less efficacious than apple powder (Frank, '37) may be a result of the difference in chemical and physical properties of commercial pectin and the pectin of apple powder (Norris and Resch, '37). Human subjects fed apple powder containing pectin 1 hour before receiving 200 cc. of milk inoculated with B. prodigiosus passed no viable bacteria of this type in the feces, while with the same subjects receiving inoculated milk but no pre-meal of apple powder, recoveries of 43 to 94% of viable B. prodigiosus in the feces were secured (Bergeim, Hanszen and Arnold, '36). In experiments on rabbits Sullivan and Manville ('38) showed that the addition of dehydrated apple to the diet definitely promoted the growth of acidophilic organisms in the intestines and raised the hydrogen-ion concentration at all levels of the intestinal tract. Besides these effects of apple constituents within the digestive tract, evidence has been reported that pectin contributes to the detoxication mechanism of the body (Manville, Bradway and McMinis, '36), apparently by providing galacturonic acid for conjugation, and that the oral administration of pectin diminishes the coagulation time of the blood (Riesser and Nagel, '35; Aragona, '36).

These desirable properties of the apple should commend it as a food in the human diet. Unfortunately it is necessary at the present time to market apples containing residues of acid lead arsenate used as an orchard spray in the control of insect pests. Although the permissible amounts of residual lead and arsenic on commercial apples are under the control of the Federal Food and Drug Administration in the case of interstate shipment and of state agencies in a number of the states, the situation is not entirely a satisfactory one, and many attempts have been and are being made to alarm the consumer and to discourage the buying of apples. Even discussions of the question by public health authorities (Hanzlik, '37) can

hardly fail to add to the disquieting effect of less responsible writers.

It occurred to the authors, on the basis of the known properties of the apple, that possibly the constituents of this fruit would adsorb the lead contained on its surface or in some way inhibit or impede its absorption from the intestinal tract and thus diminish the possibility of a harmful effect. It seemed likely that the pectic or hemicellulosic constituents might have this effect, since pectic acid solutions are precipitated by lead, calcium and barium. In fact, a quantitative method for the estimation of pectin by hydrolysis and precipitation as calcium pectate in acetic acid solution has been proposed (Carré and Haynes, '22), and lead salts are quite generally less soluble than the corresponding calcium salts.

PLAN OF EXPERIMENT

The plan of the experiment involved the feeding of twelve trios of weanling rats for the purpose of determining the lead retention on three experimental diets. The first diet was a synthetic one, planned to be complete and to equal the apple powder diets in calcium and phosphorus. The second diet contained 32% of unsprayed-apple powder, replacing agar and starch in diet 1. The third diet was like the second except that sprayed-apple powder was used in place of the unsprayed. The lead content of all three diets was the same, namely, 33 p.p.m., supplied almost entirely by the spray residue in diet 3 and by $PbHAsO_4$ in diets 1 and 2. A comparison of the lead retention promoted by diets 1 and 2 would indicate the effect of apple powder; a comparison of the retention of lead on diets 2 and 3 would indicate the relative assimilability of the lead in $PbHAsO_4$ and in the residue from lead arsenate sprays. The composition of these diets is shown in table 1.

The apple powder in diet 2 was prepared from unsprayed Huntsman apples, that in diet 3 from sprayed Jonathan apples. The whole apples including skin and core, were sliced in thin pieces and dried in a current of warm air not exceeding 70°C. in temperature. They were then placed in desiccators

for 48 hours over $CaCl_2$, transferred to stone jars in cold storage at —7°C. and ground to a powder at that temperature. The unsprayed-apple powder contained 6.28% crude fiber, 2.36% nitrogen, 2.45% ash, 0.077% calcium, and 0.84 p.p.m. of lead. The sprayed-apple powder was analyzed only for lead and was found to contain 96.4 p.p.m. These apples had purposely not been washed to remove the spray residue.

TABLE 1

Composition of the experimental diets

CONSTITUENTS	DIET 1 %	DIET 2 %	DIET 3 %
Apple powder	0	32.0 [2]	32.0 [3]
Dried extracted egg	21.5	21.5	21.5
Lard	8.5	8.5	8.5
Butter	5	5	5
Salts mixture [1]	11.5	11.5	11.5
Cod liver oil [4]	1	1	1
Wheat germ oil	0.5	0.5	0.5
Dried yeast	10	10	10
Sucrose	8	8	8
Starch	27.75	2	2
Agar	6.25	0	0
$PbHAsO_4$	54 p.p.m.	54 p.p.m.	0
Total	100	100	100
Lead	33 p.p.m.	33 p.p.m.	33 p.p.m.
Calcium	0.54%	0.51%	0.51%
Phosphorus	0.63%	0.66%	0.66%

[1] A modified Osborne and Mendel mixture, containing cobalt, zinc and copper and 76% of starch for convenience in handling. In this mixture, all of the calcium and phosphorus is in the form of $CaHPO_4$.

[2] Unsprayed-apple powder.

[3] Sprayed-apple powder (lead arsenate spray).

[4] Guaranteed to contain 3000 U.S.P. units of vitamin A and 400 U.S.P. units of vitamin D per gram.

One rat in each trio received diet 1, a second rat diet 2 and the third rat diet 3. These diets were fed in equal amounts daily within each trio but the weights of the rats in each trio were kept constant by feeding sugar in concentrated solution to the rats growing at the slower rates. The rats in each trio were matched for sex, body weight and litter

membership. They were confined individually in special cages made of tin-plated wire mesh and block tin sheets held together by lead-free solder, and particular care was taken that no contact with metallic lead was possible. With nine trios the experiment was terminated when 1 kg. of experimental diet had been consumed. Because of a temporary insufficiency of food supply, the feeding of three of the trios was terminted with the consumption of 723, 650 and 750 gm. of experimental diet, respectively.

At the end of the feeding periods, which varied in length from 89 to 173 days for the various trios, the rats were killed with ether, the body lengths measured from tip of nose to root of tail, the contents of the alimentary canal removed and the 'empty weight' determined. The carcasses were then washed to remove all possibility of lead contamination and ashed at a temperature of 500°C. preparatory to lead analysis, which was carried out according to the diphenylthiocarbazone method, using the colorimetric procedure as explained in a previous publication from this laboratory (Shields, Mitchell and Ruth, '39).

In order to determine the retention of lead by the experimental rats during the period of feeding it was necessary to sacrifice a number of check rats at the beginning of the experiment for lead analysis. Nine such rats taken from the same litters as the experimental rats and weighing approximately the same were used for this purpose.

THE RESULTS OF THE EXPERIMENT

The results of the experiment are summarized in table 2. It will be noted that results on only two rats are reported for trio 2. One of the rats, receiving diet 2, escaped from its cage before the termination of the experiment and was never recovered. The analyses of the nine check rats from which the initial lead content of the experimental rats was estimated revealed lead stores ranging from 0.026 mg. to 0.090 mg. and averaging 0.046 mg. Since there were no indications that the different litters represented in the experiment dif-

TABLE 2

The food consumption, growth data and lead analyses of the experimental rats

TRIO NUMBER	RAT NUMBER	DIETS FED	INITIAL WEIGHT	FINAL WEIGHT	GAIN	BODY LENGTH	FOOD CONSUMPTION	SUGAR CONSUMED	DAYS ON FEED	FOOD REFUSALS	INITIAL Pb CONTENT	FINAL Pb CONTENT	NET GAIN IN Pb
			gm.	*gm.*	*gm.*	*cm.*	*gm.*	*gm.*			*mg.*	*mg.*	*mg.*
1	393	1	46	223	177	22.5	723	19	91	4	0.046	0.768	0.722
	394	2	44	228	184	22.0	723	38.5	91	6	0.046	0.366	0.320
	395	3	43	232	189	22.7	723	15.5	91	2	0.046	0.500	0.454
2	396	1	37	170	133	21.0	1008	140	154	10	0.046	0.618	0.572
	398	3	40	181	141	20.1	1008	28.5	154	18	0.046	0.367	0.321
3	399	1	44	202	158	21.0	650	47	92	4	0.046	0.508	0.462
	400	2	38	204	166	20.6	650	24	92	8	0.046	0.350	0.304
	401	3	40	197	157	21.0	650	4	92	0	0.046	0.392	0.346
4	402	1	58	208	150	21.5	750	18	89	1	0.046	0.454	0.408
	403	2	58	208	150	21.6	750	29.5	89	2	0.046	0.367	0.321
	404	3	57	205	148	21.9	750	30.0	89	11	0.046	0.400	0.354
5	405	1	50	182	132	20.4	1000	35	159	9	0.046	0.506	0.460
	406	2	50	181	131	20.3	1000	9.5	159	8	0.046	0.430	0.384
	407	3	50	179	129	20.3	1000	44.5	159	23	0.046	0.326	0.280
6	408	1	42	175	133	19.8	1000	0	164	1	0.046	0.800	0.754
	409	2	42	170	128	20.6	1000	25.5	164	28	0.046	0.453	0.407
	410	3	42	170	128	20.0	1000	83.5	164	0	0.046	0.525	0.479
7	411	1	32	169	137	20.5	1000	0	173	4	0.046	0.710	0.664
	412	2	33	169	136	20.0	1000	30.5	173	13	0.046	0.367	0.321
	413	3	34	166	132	20.0	1000	75.0	173	16	0.046	0.396	0.350

8	414	1	34	162	128	20.3	1000	86.5	167	18	0.046	0.468	0.422
	415	2	34	160	126	20.5	1000	84.5	167	17	0.046	0.338	0.292
	416	3	35	162	127	20.0	1000	16.5	167	0	0.046	0.272	0.226
9	417	1	26	159	133	20.0	1000	86.0	157	1	0.046	0.586	0.540
	418	2	28	162	134	20.0	1000	45.0	157	20	0.046	0.382	0.336
	419	3	30	159	129	20.0	1000	47.5	157	16	0.046	0.358	0.312
10	420	1	28	224	196	22.7	1000	37.5	125	6	0.046	0.413	0.367
	421	2	26	192	166	22.1	1000	101	125	11	0.046	0.389	0.343
	422	3	27	232	205	23.0	1000	12	125	1	0.046	0.333	0.287
11	423	1	32	202	170	21.0	1000	115	143	8	0.046	0.628	0.582
	424	2	30	210	180	22.5	1000	75	143	8	0.046	0.534	0.488
	425	3	30	213	183	22.0	1000	6	143	4	0.046	0.310	0.264
12	426	1	30	169	139	20.3	1000	52	152	11	0.046	0.530	0.484
	427	2	30	174	144	20.5	1000	37	152	17	0.046	0.333	0.287
	428	3	30	176	146	20.0	1000	19	152	0	0.046	0.321	0.275
Averages													
Synthetic Diet (1)			38.3	187.0	148.8	20.9		53.0		6.4		0.582	0.536
Unsprayed A.P. Diet (2)			37.5	187.1	149.5	21.0		45.5		12.5		0.392	0.345
Sprayed A.P. Diet (3)			38.2	189.3	151.2	20.9		31.8		7.6		0.375	0.329

fered significantly in the amounts of accumulated lead (nor even that the heavier check rats contained more lead than the lighter), and since the check rats averaged practically the same in body weight as the experimental rats did initially, the initial lead content of all experimental rats was estimated at the same average value, 0.046 mg.

DISCUSSION

The growth of the rats within the various trios was equalized as far as body weight is concerned by adjusting the amounts of the sugar supplement given. However, since there were no consistent differences in the amounts of sugar required with the different diets, the numbers of food refusals or the attained body lengths, it is obvious that the three diets used were equally effective in promoting growth.

The lead contents of the rats at the termination of the experimental feeding, as well as the estimated amounts of lead stored during this period, reveal interesting and significant similarities as well as differences. The average lead retentions on the three diets were 0.536 mg., 0.345 mg. and 0.329 mg., respectively, equivalent to 1 to 1.5% of the lead ingested. The average difference in storage between diets 2 and 3 containing unsprayed-apple powder and sprayed-apple powder, respectively, was clearly insignificant statistically, using Student's method ('25) of analysis ($M = 0.0160$, $s = 0.0969$ and $P = 0.31$). With such a high value of P, the probability that random factors alone operated to produce the observed average difference in lead retention is far too high to be neglected. This comparison can be interpreted to mean that the lead contained in spray residue is just as readily absorbed and retained in the body as the lead in the original spray chemical, acid arsenate of lead, although weathering and leaching have undoubtedly hydrolyzed the arsenate to some extent (McDonnell and Graham, '17; Ruth and Kadow, '34); there is the possibility also of some combination of lead with the constituents of apple wax (Cohee and St. John, '34).

In parts of the country where only early summer sprays are used, a part of the lead may be in the form of lead sulfide because of the use of lime sulfur (calcium tetra and penta sulfide and thiosulfate), together with the lead arsenate, as a summer fungicide. The apples used in this experiment, however, had been sprayed with a full summer schedule of lead arsenate, so that the lead sulfide resulting from the use of lime sulfur early in the summer must have been a minor quantity, in comparison to the lead arsenate resulting from later sprays.

The average differences in lead storage between the rats on diets 1 and 2, and diets 1 and 3 were 0.191 mg. and 0.207 mg., respectively. In all trios the rat on diet 1 stored more lead than the rats on the apple-powder diets 2 and 3. The probabilities that fortuitous factors could have accounted for such consistent and marked differences are so small—less than 0.001 in both comparisons—that they may be neglected. It may be concluded, therefore, that the constituents of apples, either one or a combination of two or more, inhibit the assimilation of lead in the growing rat. In all probability this inhibition occurs within the alimentary canal and relates to absorption only. Under the conditions of this experiment the inhibition averaged 37% and for reasons stated in the introduction is probably traceable, wholly or mostly, to the pectic constituents.[2]

Thus, the apple contains within its tissues a substance or substances that exerts a considerable effect on intestinal absorbability of the lead component of the spray residue contained on its surface. Furthermore, in this experiment the ratio of lead to apple solids was 96 p.p.m. If the apple powder had been made from apples containing no more lead than the legal tolerance (0.025 grain per pound), the ratio of lead to apple solids would have been about 22 p.p.m. It seems quite likely that with this smaller ratio of lead to apple solids, the inhibiting effect of the latter on lead absorption would have been considerably greater than 37%.

[2] A solution of pectic acid, prepared from pectin by mild alkaline hydrolysis and acidified with acetic acid, will precipitate even small amounts of lead.

CONCLUSIONS

1. No evidence was obtained that the lead occurring in the spray residue on apples is any more or less, assimilable by the growing rat than the lead contained in $PbHAsO_4$, the original spray chemical.

2. There exists in the apple a substance or substances capable of depressing considerably the assimilability of lead. Under the conditions of this experiment the depression averaged 37%. Quite probably this depression in assimilability (retention in the body) relates to absorption from the intestinal tract only, and may be brought about by the pectic constituents of the apple. It seems quite likely that it would be greater with apples containing a smaller proportion of lead to solids than the apples used in this experiment, in which the proportion was more than four times that permissible in interstate commerce.

3. It would appear that feeding experiments concerned with the possible toxicity of lead in lead arsenate spray residues on fruits should involve the feeding of rations containing the fruit solids, unless it can be shown that the fruit itself does not modify the assimilability of the lead.

LITERATURE CITED

ARAGONA, G. 1936 The time of coagulation of blood after the administration of pectin to rabbits [Trans. title]. Boll. soc. ital. biol. sper., vol. 11, pp. 434–435; Chem. Abst., vol. 31 (1937), p. 1098.

BAUMANN, T., AND H. FORSCHNER-BÖKE 1934 Untersuchungen zur therapeutischen Wirkungsweise von Apfel- und Bananen-diät. Z. f. Kinderheilk., Bd. 56, S. 514–545.

BERGEIM, O., A. HANSZEN AND L. ARNOLD 1936 The influence of fruit ingestion before meals upon the bacterial flora of stomach and large intestine and on food allergins. Am. J. Dig. Dis. and Nutr., vol. 3, pp. 45–52.

CARRÉ, M. H., AND D. HAYNES 1922 The estimation of pectin as calcium pectate and the application of this method to the determination of the soluble pectin in apples. Biochem. J., vol. 16, pp. 60–69.

COHEE, R. F., AND J. L. ST. JOHN 1934 Lead ursolate in relation to fruit cleaning. Ind. Eng. Chem., vol. 26, pp. 781–782.

FRANK, E. 1937 Erfahrungen über die Behandlung von Durchfallserkrankungen in Kindesalter mit Aplona und Pektin. Deutsch. med. Wochenschr., Bd. 63, S. 1328–1329.

HANZLIK, P. J. 1937 Health hazards of chemo-enemies in contaminated foods. Sci. Mo., vol. 44, pp. 435–439.

MALYOTH, G. 1934 Das Pektin, der Hauptträger der Wirkung bei der Apfeldiät. Klin. Wochensch., Bd. 13, S. 51–54.

MANVILLE, I. A., AND E. M. BRADWAY 1936 Pectin as a detoxication mechanism. Am. J. Dig. Dis. and Nutr., vol. 3, pp. 570–572.

MANVILLE, I. A., E. M. BRADWAY AND A. S. MCMINIS 1937 The use of apple powder in the treatment of diarrheal conditions and its rationale. Canadian Med. Assoc. J., vol. 36, pp. 252–257.

MANVILLE, I. A. 1938 Use of apple powder in the prevention and cure of summer diarrheas. Arch. Pediat., vol. 55, pp. 76–84.

MCDONNELL, C. C., AND J. J. T. GRAHAM 1917 The decomposition of dilead arsenate by water. J. Am. Chem. Soc., vol. 39, pp. 1912–1918.

NORRIS, F. W., AND C. E. RESCH 1937 The pectic substances of plants. V. Further observations on the nature of pectin and pectic acid. Biochem. J., vol. 31, pp. 1945–1951.

RIESSER, O., AND A. NAGEL 1935 Über die gerinnungafördernde Wirkung saurer Substanzen, insbesondere des Petkins. Arch. exp. Path. u. Pharm., Bd. 179, S. 748–760.

RUTH, W. A., AND K. J. KADOW 1934 Spray-residue removal. Trans. Ill. State Hort. Soc., vol. 68, pp. 191–198.

SHIELDS, J. B., H. H. MITCHELL AND W. A. RUTH 1939 The metabolism and retention of lead in growing and adult rats. J. Ind. Hyg. Toxicol., vol. 2, pp. 7–23.

'STUDENT' 1925 New tables for testing the significance of observations. Metron, vol. 5, pp. 105–120.

SULLIVAN, N. P., AND I. A. MANVILLE 1938 The relationship of the diet to the self-regulatory defense mechanism. I. Hydrogen-ion concentration and bacterial flora. Am. J. Dig. Dis., vol. 5, pp. 428–432.

TODHUNTER, E. N. 1937 The nutritive value of apples. Washington Agr. Exp. Sta., Pop. Bul. 152. 32 pp.

THE ANTI-ACRODYNIC PROPERTIES OF CERTAIN FOODS [1]

HOWARD A. SCHNEIDER, J. KATHERINE ASCHAM, BLANCHE R. PLATZ AND H. STEENBOCK

Department of Biochemistry, College of Agriculture, University of Wisconsin, Madison

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Since the anti-acrodynic vitamin (anti-dermatitis vitamin, vitamin B_6) was differentiated from the other components of the vitamin B complex (Gyorgy, '35) there has been a need for a survey of the anti-acrodynic potencies of various foodstuffs. Recently with the isolation of the crystalline vitamin B_6 by Lepkovsky ('38), Gyorgy ('38) and Kuhn and Wendt ('38 a), it has become apparent that in addition to vitamin B_6 another factor is needed by the rat for the cure of dermatitis. Gyorgy ('38) after determining the effect of the crystalline vitamin commented as follows: "Even the skin effect was not regularly attained unless a further supplement corresponding to the so-called 'filtrate factor' was added." Birch ('38) produced dermatitis in rats which were supplied with ample amounts of B_6 but which lacked this accessory factor. In this laboratory acrodynia has been only temporarily relieved by crystalline vitamin B_6;[2] a severe dermatitis persisted unless a 'filtrate factor' was fed as well. From this it is apparent that any assay of anti-acrodynic potency would be in error if interpreted solely as evidence of the amount of vitamin B_6 present. However, irrespective of the composite nature of the anti-acrodynic factor, information regarding its

[1] Published with the approval of the director of the Wisconsin Agricultural Experiment Station.

[2] Dr. S. Lepkovsky kindly furnished us a sample of the crystalline vitamin.

distribution as determined by biological assay should be of value. In anticipation of a similar need, Wilson and Roy ('38) have recently published a survey of the anti-dermatitis factor in Indian foodstuffs.

EXPERIMENTAL

The technic of assay was the same as that outlined by Quackenbush, Platz and Steenbock ('38). Weanling rats, reared by mothers on a potato diet low in the anti-acrodynic factor, were transferred to a basal diet of the following composition: cerelose (glucose) 78, casein (alcohol extracted) 18, Wesson salts 4. Each rat was given in addition a daily supplement of 5 micrograms calciferol and 10 micrograms β-carotene in 1 drop of the liquid portion of hydrogenated coconut oil, plus 20 micrograms riboflavin and 10 micrograms thiamin hydrochloride in 1 drop of N/50 acetic acid. The only components of the B complex which were supplied by this diet (diet V) were riboflavin and thiamin. Acrodynia was produced in 4 to 5 weeks.

When the acrodynia had developed to such a degree that the paws were erythemic, swollen and denuded, and the lips were fissured, swollen and denuded the ration was supplemented with the material to be assayed. These supplements were fed in small glass dishes. Unless otherwise noted vegetables and fruits were fed fresh, and legumes, seeds and cereals were fed air dried. Other materials were fed fresh or dried as indicated in table 1.

When a curative effect became evident, the mouth symptoms were the first to clear up, the swelling and soreness being reduced gradually. The forepaws healed before the hind paws with a disappearance of the erythema and the swelling. New fur started to grow on the healed areas. A scaliness usually persisted on the paws and tail for several weeks after the disappearance of the other symptoms. The supplement was assumed to be curative if at the end of a 3-week period the lips, eyes and ears were normal in appearance, and the paws and tail were free from all symptoms except a slight scaliness.

TABLE 1

The anti-acrodynic potency of foods

	MINIMUM CURATIVE LEVEL (MG./DAY)	MAXIMUM FAILING LEVEL (MG./DAY)	UNITS PER 100 GM.	WILSON AND ROY VALUES (RECALC.)
Green leafy vegetables				
1. Lettuce	4000	1500	25	77
2. Spinach	1500	1000	66	53
Vegetables				
3. Tomato	4000	1500	25	42
4. Potato (dried)	500	300	200	
(calc. as fresh)	2500	1500	40	75
5. Carrot	4000	1500	25	
6. Beet (red)	7500	5000	13	
Fruits				
7. Banana	1500	500	66	
8. Orange	6000	5000	16	23
9. Apple	5000	4000	25	57
Legumes, seeds, cereals				
10. Alfalfa leaves	170	40	600	
11. Beans (navy)	250	20	400	
12. Peanuts	60	30	1660	
13. Soybeans	80	60	1250	
14. Cornmeal	250	200	400	
15. Whole wheat bread	250	200	400	
16. Oat meal	300	250	330	
17. Flaxseed	100	80	1000	
18. Rice polishings	200	150	500	
19. Wheat germ	80	60	1250	
Animal products				
20. Egg white (boiled, dried)	...	500	...	
21. Egg yolk (boiled, dried)	40	10	2500	
22. Whole milk	2500	...	40	
23. Skim milk	7000	5000	14	
24. Cheese (Cheddar)	400	300	250	
25. Beef muscle (raw, dried)	800	500	125	
26. Beef muscle (roasted, dried)	800	500	125	
27. Haddock (dried)	500	150	200	
28. Pork liver (dried)	200	80	500	
Fats (animal)				
29. Beef tallow	300	20	330	
30. Butterfat	500	200	200	
31. Chaulmoogra oil	...	40	...	
32. Cod liver oil	...	40	...	
33. Lard	40	...	2500	
Fats (plant)				
34. Coconut oil	200	100	500	
35. Coconut oil (hydrogenated)	...	200	...	
36. Linseed oil (comm.)	40	20	2500	
37. Linseed oil (crude)	40	20	2500	
38. Linseed oil (refined)	...	40	...	
39. Linseed oil (hydrogenated)	...	100	...	
40. Peanut oil (ether ext.)	20	10	5000	
41. Peanut oil (benzine ext.)	20	...	5000	
42. Peanut oil (crude)	40	20	2500	
43. Rice oil (comm.)	40	20	2500	
44. Rice oil (ether ext.)	20	...	5000	
45. Soybean oil (ether ext.)	10	...	10000	
46. Soybean oil (ether ext.)	15	...	7500	
47. Tung oil	...	40	...	
48. Cottonseed oil (comm.)	10	5	10000	
49. Corn oil (comm.)	5	3	20000	
50. Wheat germ oil (comm.)	4	2	25000	
51. Wheat germ oil (ether ext.)	7	...	15000	
Miscellaneous				
52. Yeast (dried)	250	200	400	506

If the amount of supplement proved to be insufficient to effect a cure, a larger amount was fed to another rat. If a supplement effected a cure rapidly, progressively smaller amounts were fed until failure resulted. For comparison of the relative potencies of the materials examined a unit of anti-acrodynic potency was defined as the minimal daily supplement which would cure an acrodynia of moderate severity in 3 weeks. Wilson and Roy ('38) in adopting a similar unit used 1 week as the period of assay.

DISCUSSION

Although in most cases both the minimal curative and the maximal failing doses are reported (table 1), assay data were obtained for many of the materials at levels above or below these limits. For purposes of comparison the assays of Wilson and Roy ('38), converted into our units, have been included for those materials of which we had corresponding data. In performing this conversion it was arbitrarily assumed that an animal cured in 1 week by a certain daily portion (Wilson and Roy unit) would be cured in 3 weeks by a daily dose of one-third of that portion. The agreement for those materials which could be compared in this manner is fairly satisfactory, certainly within the limits of errors of the assumed 'conversion.'

In the interpretation of results allowance must be made for the possible influence of members of the vitamin B complex other than those directly involved in the cure of the acrodynia. In the feeding of the supplements it was noted that apart from the healing of symptoms varied rates of growth were obtained. What effect these varied rates of growth had on the requirements for the anti-acrodynic factor cannot be estimated here. The data obtained, therefore, can be used for comparative purposes only.

It is apparent that cereals are richer in anti-dermatitis potency than fruits and vegetables. Meat and fish occupy an intermediate position. This generalization is also the conclusion of Wilson and Roy for Indian foodstuffs of similar

classification. However, these materials are relatively poor sources of the anti-dermatitis factors when compared with the potencies of certain fats. Thus soybean, cottonseed, corn and wheat germ oils are among the most potent substances assayed. Wheat germ oil has a potency approximately sixty times that of maize. It is interesting to note that yeast is not a rich source of the anti-acrodynic factor as it ranked with the cereals in potency and was easily exceeded by many of the fats. It is also interesting to note that lard, which is a common ingredient of many experimental rations is actually six times as potent as yeast.

Whether the potency of fats is to be attributed to an actual B_6 content cannot be answered at the moment although Kuhn and Wendt ('38 b) have reported that the B_6 molecule contains two alcoholic and one phenolic group which would make an ester form of the vitamin at least a possibility. However, Birch ('38) and Quackenbush, Platz and Steenbock ('38) have traced the potency of fats to the unsaturated fatty acid fractions. Salmon ('38) likewise obtained cures with certain fats and fatty acids. Birch suggested that the unsaturated fatty acid factor is necessary for a cure of dermatitis in addition to B_6 on the basis that where B_6 failed to cure alone, the addition of fat made a cure possible.

Whatever may be the mode of action of anti-acrodynic fats it is true that they constitute an extraordinarily potent source of anti-acrodynic activity. Therefore these fats must be included in any survey of the anti-acrodynic potency of foodstuffs.

The evaluation of these findings for human nutrition is impossible at the moment since the role of vitamin B_6 in human nutrition is as yet undemonstrated. Indeed, apart from the rat, the only other species for which vitamin B_6 has been indicated to be essential are the dog (Fouts, Helmer, Lepkovsky and Jukes, '38) and the pig (Chick, Macrae, Martin and Martin, '38).

SUMMARY

Fifty-two materials have been assayed for their anti-acrodynic activity using the rat as the experimental animal. Fruits and vegetables were found to be poor sources, fish and meat fair, seeds, legumes and cereals relatively rich and certain vegetable fats extremely potent sources of anti-acrodynic activity.

LITERATURE CITED

BIRCH, T. W. 1938 The relation between vitamin B_6 and the unsaturated fatty acid factor. J. Biol. Chem., vol. 124, p. 775.

CHICK, H., T. F. MACRAE, A. J. P. MARTIN AND C. J. MARTIN 1938 The water soluble B-vitamins other than aneurin (vitamin B_1), riboflavin and nicotinic acid required by the pig. Biochem. J., vol. 32, p. 2207.

FOUTS, P. J., O. M. HELMER, S. LEPKOVSKY AND T. H. JUKES 1938 Production of microcytic hypochromic anemia in puppies on synthetic diet deficient in rat anti-dermatitis factor (vitamin B_6). J. Nutrition, vol. 16, p. 197.

GYORGY, P. 1935 The differentiation of lactoflavin and the 'rat anti-pellagra' factor. Biochem. J., vol. 29, p. 741.

———— 1938 Crystalline vitamin B_6. J. Am. Chem. Soc., vol. 60, p. 983.

KUHN, R., AND G. WENDT 1938 a Über das anti-dermatitische Vitamin der Hefe. Ber. Chem. Ges., Bd. 71, S. 780.

———— 1938 b Über die funktionellen Gruppen des Adermins. Ber. Chem. Ges., Bd. 71, S. 1534.

LEPKOVSKY, S. 1938 Crystalline factor I. Science, vol. 87, p. 169.

QUACKENBUSH, F. W. B. R. PLATZ AND H. STEENBOCK 1939 Rat acrodynia and the essential fatty acids. J. Nutrition, vol. 17, p. 115.

SALMON, W. D. 1938 The effect of certain oils in alleviating localized erythematous dermatitis (acrodynia or vitamin B_6 deficiency) in rats. Proc. Am. Soc. Biol. Chem., J. Biol. Chem., vol. 123, p. civ.

WILSON, H. E. C., AND G. K. ROY 1938 The flavine and vitamin B_6 (anti-dermatitis) content of Indian foodstuffs. Indian J. Med. Res., vol. 25, p. 879.